AN IN VITRO EVALUATION OF BACTERIAL LEAKAGE USING 3 DIFFERENT RETROGRADE FILLING MATERIALS - A CONFOCAL LASER SCANNING MICROSCOPIC STUDY

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BRANCH – IV

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ENDORSEMENT BY THE H.O.D. PRINCIPAL / THE HEAD OF THE INSTITUTION

This is to certify that **Dr. POOJITHA VISWANATH,** Post Graduate student (2015–2018) in the Department of Conservative Dentistry and Endodontics, K.S.R. Institute of Dental Science and Research, has done this dissertation titled **"AN IN VITRO EVALUATION OF BACTERIAL LEAKAGE USING 3 DIFFERENT RETROGRADE FILLING MATERIALS - A CONFOCAL LASER SCANNING MICROSCOPIC STUDY"** under our guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University**, Chennai – 600 032 for **M.D.S.,** (Branch – IV) **CONSERVATIVE DENTISTRY AND ENDODONTICS** degree examination.

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INTRODUCTION

INTRODUCTION

The major goal of root canal treatment is to clean and shape the root canal system and seal it in three dimensional way to prevent reinfection of the tooth. The complexity of root canal makes it difficult to clean completely especially in the apical region. The apical third comprises of lateral canals, accessory canals, apical delta, isthmus, ramifications. The communication between the main body of the root canal and the periodontal ligament space are through the channels of lateral canals at the apical third and the accessory canals which are found in few millimetres within the apex of the root, forms apical delta. The supply of nutrients to bacteria located in these ramifications and apical deltas will remain unaltered even after root canal therapy, which leads to constant presence of bacteria and necrotic tissue remnants in the apical portion.[1] These irritants from the root canal egress in to the radicular tissues results in apical periodontitis , leads to periapical lesions. [2]

The factors which remain challenge for cleaning and disinfection of the root canal space in nonsurgical endodontic treatment are biofilm resistance organisms, poor penetration of the medicament/irrigant, low concentration of irrigant, short exposure time, minimal volume, and poor exchange of irrigants in the apical portion. Moreover biofilms which are attached to the apical root surface (extraradicular biofilms) is considered as a possible cause of post-treatment apical periodontitis, results in failure of root canal treatment.[3]

Surgical endodontic treatment is indicated for teeth with post treatment apical periodontitis, when a nonsurgical retreatment does not give desired results. The main goal of the surgical endodontic treatment is to prevent the bacterial invasion and their byproducts from the root canal system into the periradicular tissues by sealing it with adequate and efficient root end filling material.[4]

According to Gartner and Dorn an ideal material to seal the root-end cavities should prevent leakage of microorganisms and their by-products into the periradicular tissues. It should be non-toxic, non-carcinogenic, biocompatible with the tissue fluids, dimensionally stable, sealing ability not affected by moisture presence ,easy to use , radio-opaque, and to be easily recognized on the radiograph.[5] A wide variety of materials have been used as retrograde materials such as gold-foil, silver posts, titanium screws, tin posts , amalgam (with and without bonding agent), cements like zinc polycarboxylate, zinc phosphate , glass Ionomer cements, mineral trioxide aggregate, calcium phosphate and bone cement, composite resin (with and without bonding agent) and gutta-percha.[6]

Mineral Trioxide Aggregrate (MTA Plus™ - Prevest denpro Limited) is commonly used for root-end filling material. MTA, is biocompatible, bacteriostatic agent with better sealing properties. It is composed of tricalcium silicate, dicalcium silicate, bismuth oxide and small proportions of tricalcium aluminate and calcium sulphate. Recently new materials are introduced which is more biocompatible which act as both osseoinductive and osseoconductive.

Biodentine™ (Septodont, St. Maurdes Fossıs, France) is a relatively new material introduced as a dentine substitute. Biodentine powder is mainly composed of supremely pure tricalcium silicate, which regulates the setting reaction, calcium carbonate (filler) and zirconium dioxide (radiopacifier). The liquid contains calcium chloride (setting accelerator), water reducing agent (super-plasticizer) and water. The super-plasticizer reduces the viscosity of the cement and improves handling characterictics.[7]

Brasseler USA® EndoSequence® Root Repair Material™ (ERRM) is a ready-to-use, premixed bioceramic-based material composed of calcium silicates, zirconium oxide, tantalum pentoxide, calcium phosphate monobasic and filler agents. It is available as

premixed syringeable paste or putty form. BC-RRM is biocompatible which exhibits antibacterial and antifungal properties.[8]

Enterococcus faecalis are frequently isolated bacteria from obturated root canals of teeth that exhibit chronic periapical pathology. They are gram - positive and facultative anaerobes, in which *E. faecalis* play an important role in bacterial biofilm formation. It is a non-motile, facultative anaerobic bacterium, ability to withstand alkaline conditions and glucose starvation which causes persistent infections.[9] Its prevalence ranges from 24% to 77% in secondary infection.[10]

Various methods are used to assess the quality of root-end filling materials like degrees of dye penetration, bacterial penetration, electromechanical ways and fluid filtration technique[11] in which bacterial leakage studies are more reliable tests whereas dye leakage methods do not simulate the conditions in the oral cavity, which require long periods of observation time, and are sensitive .The bacterial leakage test reflects clinical reality, since it uses bacteria, which are etiologic agents of apical periodontitis .[12]

Confocal laser scanning microscopy (CLSM) is the traditional microbiological histological standard electron microscopy which provide a direct and quantitative information about the presence and distribution of bacteria inside the dentinal tubules and in the total circumference of the root canal walls. It assess both viable and dead bacteria through live/dead staining method.[13]

The purpose of this study was to compare the efficacy of MTA Plus™, Biodentine ™, EndoSequence® Root Repair Material™ as root end filling in bacterial leakage and confirming it in Confocal Laser scanning microscope.

AIM & OBJECTIVES

AIM

To compare the bacterial leakage of 3 different retrograde filling materials using confocal laser scanning microscopy.

OBJECTIVES

The main objectives was to

- Evaluate the turbidity obtained from MTA Plus™, Biodentine™, *EndoSequence® Root Repair Material*™ (ERRM) in lower apparatus by using ELISA Reader
- Evaluate the distribution of *Enterococccus faecalis* in root canal by sectioning it and observing under confocal laser scanning microscopy.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Root End Filling Materials

1. **Jingzhi Ma et al (2011)** evaluated the biocompatibility of 2 root-end filling materials, Endosequence Root Repair Material Putty (ERRM Putty) and Paste (ERRM Paste) and compared them with gray mineral trioxide aggregate (MTA). In this study Cell viability was evaluated by receiving human gingival fibroblasts which were incubated for 1, 3, and 7 days with extracts of varying concentrations from materials set for 2 days or 7 days in method of methyl-thiazol-tetrazolium (MTT) assay. Under scanning electron microscope, cell adhesion assay were examined directly after setting, after incubation in cell culture medium for 7 days, and after incubation in gingival fibroblast suspension at a density of 5×10^4 cells/well for 2 and 7 days. By energy dispersive analysis the constituents of crystals formed on surface of materials were determined. They have concluded, ERRM Putty and ERRM Paste displayed similar biocompatibility in comparison with MTA. [14]
2. **Lovato et al (2011)** compared antimicrobial activity of Endosequence putty and paste form with ProRoot MTA against clinical isolates of *Enterococcus Faecalis*. A vertically positioned 96 well microtiter plate was taken in which a section of 3 mm diameter and 1 mm thickness of respective materials (n=10) were coated. A 10µl of bacterial suspension were placed on the surface of the materials and uncoated materials acted as negative controls. After 24 – 48 hrs of aerobic incubation, CFU's were enumerated and calculated. It was concluded that Endosequence Root Repair Material (ERRM), both putty and paste forms and ProRoot MTA showed similar antimicrobial activity. [15]
3. **Kokate et al (2012)** evaluated the microleakage of three root end filling materials Mineral Trioxide Aggregate (MTA), Glass Ionomer Cement (GIC) & Biodentine™

using dye penetration. In this study thirty extracted single rooted lower mandibular premolars were taken, and root end cavities were prepared at a depth of 3mm with ultrasonic microsurgical tips. The teeth were divided into three groups and they were subdivided into two subgroups (n=5) and were contaminated with blood or artificial saliva. Following the Group A, B was filled with ProRoot MTA, after that Group C, D was filled with Biodentine™ and then Group E, F filled with Injectable Glass Ionomer composite respectively. Samples were immersed in 50% AgNO3 dye and were examined under stereomicroscope at 30x magnification. Statistical analyses were done and there is no significant difference between the sealing ability of MTA and Biodentine™ in blood and saliva contaminated cavities. They concluded that , both MTA and Biodentine™ showed significant difference in sealing ability in comparison with Injectable Glass Ionomer composite. When compared with MTA in blood and saliva contaminated cavities , Biodentine™ has similar sealing ability.[16]

4. **Attik et al (2014)** evaluated the biocompatibility of Biodentine™ and White ProRoot mineral trioxide aggregate (MTA) with MG63 osteoblast-like cells and to distinguish the cement surface in invitro . A direct contact model was established for MG63 osteoblast-like cells with cements was used for 1, 3 and 5 days. The investigation carried out was (i) cement surface characterization by atomic force microscopy (AFM), (ii) cell viability by MTT assay, (iii) protein amount quantification by Bradford assay and (iv) cell morphology by SEM. Analysis of variance (ANOVA) with a repetition test were done for statistical analyses. They have concluded that the biocompatibility of Biodentine™ to bone cells was comparable to MTA.[17]
5. **Shokouhinejad et al (2014)** compared the marginal adaptation of Endosequence root repair materials (paste and putty form) and MTA as root end filling material. Thirty six extracted tooth were chemomechanically prepared and obturated with gutta-percha

and AH- 26 sealer. At 3 mm, roots were resected and cavity was prepared with ultrasonic retrotip. Each group(n=12) were filled with MTA,ERRM (paste and putty) form respectively .Using scanning electronic microscope , gaps between interphase and dentin were measured at transverse, longitudinal , and overall gaps . Kruskal Wallis test were used for statistical analysis. There is no significant difference between three groups in transverse sections. ERRM Paste formed larger gaps in longitudinal section when compared to ERRM Putty and MTA .They concluded that in overall gaps ERRM Putty and Paste were comparable to MTA , whereas in longitudinal sections due to superior adaptation property ERRM Putty is more suitable than ERRM Paste.[18]

6. **Wang et al (2014)** investigated the microhardness and microstructural features of Mineral trioxide aggregate (MTA), Endosequence Root Repair Material Putty (ERRM Putty) and Endosequence Root Repair Material Paste (ERRM Paste), after exposure to a range of acidic environments in comparison with intermediate restorative material (IRM). Twenty specimens of each group were taken , among them fifteen specimens per each material were randomly subdivided into three groups (n = 5). They were exposed to butyric acid which was buffered at three different pH levels (5.4, 6.4 and 7.4) for 7 days and as a control group the remaining five specimens were exposed to distilled water. After 7-days the surface microhardness after exposure either to acid or to water was measured at 37 °C. Using a scanning electron microscope (SEM) the morphology of the internal microstructure was observed. Two-way univariate analysis of variance (ANOVA) was applied to evaluate the Vickers microhardness value (VHN).The results showed that microhardness value for ERRM paste and MTA reduced in acidic environment. They have more porous and less crystalline microstructure.[19]

7. **Gomes-Cornelio et al (2015)** compared the bioactivity of Biodentine™ (BIO, Septodont), MTA Plus (MTA P, Avalon) and calcium silicate experimental cement (CSC) with resin (CSCR) associated with zirconium (CSCR ZrO2) or niobium(CSCR Nb2O5) oxide as radiopacifiers. In this study, osteoblastic cell response for mineralized tissue repair, were assessed in test materials through viability (MTT), cell proliferation gene expression of alkaline phosphatase (ALP) osteogenic marker by real-time PCR (RT-qPCR), ALP activity assay by using human osteoblastic cells (Saos-2) in test materials and additionally alizarin red staining (ARS) were to detect mineralization nodule deposition in osteogenic medium. Unexposed cells acted as the control group(C). Statistical analysis was carried out using ANOVA and the Bonferroni post-test ($P < 0.05$).The results showed that the MTA Plus , Biodentine™ and CSCR ZrO2 groups had the highest viability rates and velocity of proliferation whereas the CSCR Nb2O5 group produced more mineralized nodules.[20]
8. **Amir T. Moinzadeh et al (2016)** conducted study to distinguish and evaluate the interaction of EndoSequence BC RRM putty (Brasseler USA, Savannah, GA) in contact with blood and simulated body fluid. The materials were chosen from various medium such as water, Hank's balanced salt solution, and heparinized whole blood and also an explanted material from a failed root-end surgery was performed. The features of EndoSequence BC RRM putty was done by scanning electron microscopy, energy dispersive spectroscopy, and X-ray diffraction analysis before setting and after contact with medias. The results revealed that the hydration of EndoSequence RMM putty is affected by the environmental conditions and consequentially the interaction of the material with the environment. However, in vitro environment does not represent the clinical situation because at the end calcium carbonate was formed

because of the presence of carbon dioxide in vivo rather than the hydroxyapatite reported in in vitro studies.[21]

9. **Huseyin Akcay et al (2016**) assessed the bond strength of root-end placed MTA and Biodentine™ (Septodont, Saint Maur des Fossés, France) in the absence/presence of blood contamination. In this study forty-eight single-rooted maxillary incisors were taken and root-end were resected, apical preparation was done using ultrasonic retro-tips, the specimens were randomly divided into two groups and filled with retrograde material respectively. The specimens were then subdivided into two groups according to storage condition (absence/presence of blood) (n = 12). A size of 2.0 ± 0.1 mm slices, were obtained and push-out tests were performed. The failure mode was examined under a stereomicroscope for each slice .Statistical analysis were done using two-way analysis of variance and Tukey's *post hoc* test for multiple comparisons . They have concluded that Biodentine™ had better bond strength values compared to MTA, and the the presence of blood did not effect the bond strength of both MTA and Biodentine™ as root-end filling materials.[22]
10. **Hernán Coaguila-Llerena et al (2016)** evaluated the cytotoxicity of three root-end filling materials : MTA Angelus®, EndoSequence Root Repair Material Putty® and Super EBA on human periodontal ligament fibroblasts . In order to evaluate the cytotoxicity of the three extracts from the root-end filling material a primary culture of human periodontal ligament fibroblasts was previously obtained after 2 and 7 days of setting of the materials. Using the methyl-thiazol-tetrazolium (MTT) colorimetric assay , serial dilutions of these extracts (1:1, 1:2, 1:4 and 1:8) were evaluated at 1, 3 and 7 days. In negative control group, cell viability was confirmed as 100%. Statistically analyses were done with t-test, ANOVA and Kruskal-Wallis test at a significance level of 5%. For 2-days setting Cell viability of MTA Angelus® was

superior ($p<0.05$), when compared with the other two root-end fillings. In 7-days setting there were no statistically significant differences between MTA Angelus® and EndoSequence Root Repair Material Putty®. Among them Super EBA® showed the lowest percentage of cell viability at higher dilutions ($p<0.05$). Therefore, they concluded that MTA Angelus® and EndoSequence Root Repair Material Putty® were less cytotoxic in the highest dilution (1:1) when compared with Super EBA®.[23]

11. **Nagesh et al (2016)** compared the sealing ability of mineral trioxide aggregate (MTA) and EndoSequence root repair material with chitosan and carboxymethyl chitosan (CMC) as retrograde smear layer removing agents and examined using scanning electron microscopy (SEM).Forty human single rooted teeth were prepared and obturated and then roots were resected apically and retrograde cavities were done. Samples were divided into four groups, (N = 10) as Group I chitosan with EndoSequence, group II chitosan with MTA, group III CMC with EndoSequence, and Group IV CMC with MTA. Further all the samples were sectioned longitudinally, and marginal adaptation were analysed under scanning electron microscope. Statistical analysis were done with Kruskal-Wallis and Mann-Witney analysis tests. They concluded that EndoSequence as retrograde material showed better marginal sealing ability.[24]
12. **Seedat et al (2016)** compared microleakage between three calcium silicate cements (ProROOT MTA, MTA PLUS™, BIODENTINE™) and permite amalgam. In this study 120 single rooted teeth were endodontically treated, 3mm of apical portion were resected and root end cavity was prepared in each sample. They were divided into 4 groups (n=30) and filled with respective samples and were submerged in india ink for 48 hours. Horizontal sectioning was done in 1mm increments from the apical end and dye penetration was analysed in stereomicroscope. They have concluded that

calcium silicate cements showed better sealing ability when compared with permite amalgam. [25]

13. **Miriam Zaccaro Scelza et al (2017)** assessed response of human primary osteoblasts to Biodentine™ using MTA Angelus™ a reference material, in an *in vitro* study. They also analyzed three different cell viability parameters, namely mitochondrial activity, membrane integrity, and cell density. After mixing Biodentine™ and MTA extracts were prepared by incubation on culture media for 24 hours or 42 days. For 24 hours, Primary human osteoblasts were exposed to extracts at 37°C with 5% CO2, and cell viability was evaluated by the XTT, NRU, and CVDE assays. The results revealed that both materials induced cell viability levels higher than 70% when extracted for first 24 hours .When cells were exposed to extracts with increased time MTA presented significant cytotoxic effects ($p < 0.05$) in comparison to the control and MTA at 24 hours . After 42 days, the XTT assay identified a significant reduction in cell viability by Biodentine™ when compared to the control ($p<0.05$), but 70% viability cutoff were attained for biocompatible materials. Hence it can be concluded that Biodentine™ is cytocompatible with human primary osteoblasts, indicating its adequacy in direct contact with bone tissue.[26]

Methodology

Bacterial leakage

1. **Torabinejad et al (1993)** assessed the sealing ability of amalgam, super EBA, and a mineral trioxide aggregate under fluorescent dye leakage and confocal microscope. 30 single rooted canals were cleaned, shaped and obturated with gutta percha and root canal sealer. After retrograde filling apical 3 mm of root were cut and exposed to dye for 24 hrs . The results showed that MTA had less leakage than amalgam and super EBA.[2]
2. **Torabinejad et al (1995)** conducted bacterial leakage study in which forty eight tooth were taken , root end cavities were filled with amalgam, Super-EBA, IRM, or MTA. Four root-end cavities were filled with thermoplasticized gutta-percha without a root canal sealer (+ control), and another four were filled with sticky wax covered with two layers of nail polish (- control). A tenth of a microliter of broth containing *S. epidermidis* was placed into the root canal of 46 teeth (40 experimental, 3 positive, and 3 negative control groups). In addition, the root canals of two teeth with test root-end filling materials and one tooth from the positive and negative control groups were filled with sterile saline and kept for 90 days. Among these MTA showed less leakage than other root end filling materials.[27]
3. **Fiscer et al (1998)** proved Mineral Trioxide Aggregate to be a most effective root-end filling material against penetration of *S. marcescens.* In this study fifty-six, single-rooted human teeth were cleaned and shaped, out of which 48 root-end cavities were ultrasonically prepared to a 3 mm depth and filled with amalgam, super EBA,IRM, and MTA respectively. Four root-end cavities were filled with thermoplasticized gutta-percha without a root canal sealer and served as positive controls. Another four root-end cavities were filled with sticky wax covered with

two layers of nail polish and served as negative controls, apparatus were kept for 101 days. The results showed that four samples of MTA did not exhibited any leakage. MTA was most effective root end material against S.marcescens.[28]

4. **Adamo et al (1999)** conducted study on bacterial microleakage model in which sixty single rooted teeth were taken which were divided into five groups MTA, Super EBA,TPH, composite resin with Probond dentine bonding agent , Dispersealloy amalgam with and without ProBond, and positive and negative control groups respectively. The samples were inoculated with a suspension of *Stretococcus salivarius* and observed for 12 weeks. It was concluded that there was no statistically significant differences in rate of microleakage among the five groups even after 12 weeks.[29]
5. **Maltezos et al (2006)** compared root-end sealing of the Resilon/Epiphany system (RES) with Pro Root MTA and Super-EBA using a bacterial leakage system. Fifty-five extracted teeth were divided into three experimental groups containing 15 samples each and 5 samples each in control group. *Streptococcus salivarius* was introduced coronally and the apical 4 mm were immersed in BHI culture medium with phenol red indicator. Bacterial leakage was monitored every 24 h for 4 wk. Gram stains were performed to confirm that the recovered bacteria were *S. salivarius.* There was no statistical difference between Resilon/Epiphany system and MTA.[30]
6. **Kazem et al (2010)** did study on comparison on both bacterial and dye leakage of different root end filling materials. Fifty six intact tooth were taken and divided into 4 groups containing 12 teeth each and 2 groups containing 3 teeth each. The materials used in the study were amalgam, Root Mineral Trioxide Aggregate (Root MTA), White ProRoot MTA (WMTA), and calcium enriched mixture (CEM)

cement. Bacterial leakage was investigated in Trypticase Soy Broth (containing *Enterococcus faecalis*) after 70 days and 1% methylene blue dye leakage was assessed after 72 hours. Complete dye leakage was checked using stereomicroscope (×40). There was no significant measure of agreement between dye and bacterial penetration along root-end fillings. CEM cement was not significantly different from currently used retrofilling materials.[31]

7. **Yildrium et al (2010)** confirmed that thickness of root end material has no influence in bacterial leakage and also smear layer removal may not be necessary in root end filled cavities with MTA. In this study seventy single rooted teeth were used and cleaning and shaping were done to a size of 50 .The teeth were randomly divided into 4 groups,15 samples in experimental groups and 5 positive and 5 negative control samples . In the first and second groups, the teeth were irrigated with only 5.25% NaOCl. In the third and fourth groups, the teeth were irrigated with 17% EDTA and 5.25% NaOCl to remove the smear layer. The cavities were prepared to a depth of 3 mm in first and third groups, and 5 mm in second and fourth groups and all samples were filled with MTA. The samples were subjected to dual chamber method where apical 3 – 4 mm were immersed in brain heart infusion medium and coronal access were inoculated with a suspension of *Enterococcus faecalis* for 4 weeks. The results revealed that thickness of root end cavity had no influence in bacterial leakage with MTA. Removal of smear layer may not be necessary when filled with MTA.[32]
8. **Uma Nair et al (2011)** compared the sealing ability of white MTA and EndoSequence Bioceramic Root-end Repair (BCRR). In this study forty single rooted teeth were taken, obturated , root end resection were done and retrofilled with 2 materials. : white ProRoot MTA (WMTA) (n= 15) and BCRR (n =15).

Positive (n=10) and negative (n=10) received no retrofill and were used as controls. All groups received *E. faecalis* in a created reservoir coronally and finally colony forming units were counted .The results were analyzed with 1-way analysis of variance and concluded that BCRR was equivalent to WMTA as a root end filling material.[33]

9. **Kazem et al (2013)** assessed bacterial and dye microleakage of white and grey mineral trioxide aggregate (WMTA and GMTA), Portland cement and calcium enriched mixture (CEM) cement as root end filling material,. Fifty four single rooted teeth were taken and were randomly divided into four study and two control groups. Samples were instrumented and filled with gutta-percha and AH26 sealer. Root ends were resected 3 mm above the rootend and deep cavities were prepared and they were filled with each material. *Enterococcus faecalis* and methylene blue dye were used for determination of bacterial and dye leakage respectively. He concluded that CEM also provides the leakage results comparable to WMTA and there was poor agreement between dye and bacterial leakage methods.[34]
10. **Antunes et al (2015)** compared the sealing ability of mineral trioxide aggregate (MTA) and EndoSequence BioCeramic Root Repair Material- Fast Set (BC-RRM) Putty invitro using a novel bacterial nutrient leakage model, in which information on whether or not intracanal bacteria are receiving nutrients from serum via leakage channels are provided. Sixty single rooted teeth were instrumented and were subjected to root-end resection and ultrasonic preparation. In experimental groups, cavities were filled with test materials and in positive control group, warm Gutta-percha and no sealer and in negative group the entire resected surface was covered with varnish. After sterilization root canal were filled with *Enterococcus faecalis* , root end were immersed in foetal bovine serum ,colony forming units were counted

after 30 days of incubation. The results showed that MTA and BC-RRM putty had similar sealing ability. [8]

11. **Medeiros et al (2016)** evaluated the sealing ability of MTA, CPM, MBPc (Moraes and Berbert Portland cement) using *Enterococcus faecalis* leakage model. In this study seventy single rooted teeth were taken and instrumented, and the root ends were resected for 3mm. Cavities were filled with test materials, coronally *Enterococcus faecalis* were incubated and the apical portion was immersed with BHI medium with phenol red indicator. The apparatus were monitored for 4 weeks. The results concluded that the epoxy resin-based cement MBPc had lower bacterial leakage when compared with the calcium silicate-based cements MTA and CPM.[35]
12. **Shahriari et al (2016)** compared the apical sealing ability of mineral trioxide aggregate (MTA), intermediate restorative material (IRM) and calcium-enriched mixture (CEM) cement using a bacterial leakage model. Eighty three single-rooted human teeth were decoronated and root canals were prepared using the step-back technique. Root end resection was done at 3 mm and cavity was prepared using an ultrasonic instrument. The cavities were filled with respective test materials and the roots were inserted into cut-end microtubes. After sterilization with ethylene oxide, microtubes were placed in sterile vials containing Brain Heart Infusion (BHI) broth and incubated at 37°C. *Enterococcus faecalis* suspension compatible with 0.5 McFarland standard (1.5×108 cell/ ml) was inoculated and procedure was continued for 70 days. The results showed that the three tested root end filling materials had equal sealing efficacy for preventing bacterial leakage.[36]
13. **Eskandarinezhad et al (2017)** compared the sealing efficacy of mineral trioxide aggregate with and without nanosilver as root end filling under bacterial leakage model. Seventy canine teeth were prepared and obturated , the root-end cavities

were prepared with ultrasonic retrotips after root end resection. Teeth were randomly divided into 4 groups containing two experimental groups (n=30) and two negative and positive controls (n=5). In group 1 and 2, root-end cavities were filled with MTA and MTA with nanosilver (by 1% weight) respectively. Bacterial leakage assessment was carried out with *Enterococcus faecalis* species for 90 days and the results concluded that adding nanosilver to MTA decreased its sealing ability.[37]

Dye with CLSM

1. **RaviChandra P.V. et al (2014)** assessed the marginal adaptation of three root-end filling materials such as Glass ionomer cement, Mineral trioxide aggregate and Biodentine™. .In this study thirty human single-rooted teeth were resected 3 mm from the apex. Root-end prepration were done using an ultrasonic tip and consequently filled with one of the following materials - Glass ionomer cement (GIC), Mineral trioxide aggregate (MTA) and a bioactive cement Biodentine™. With 1 mm thick transversal sections, the apical portions of the roots were sectioned and area of gaps and adaptation of the root-end filling materials with the dentin were determined using Confocal laser scanning microscopy (CLSM). Statistical analysis were done using the Post hoc test, a multiple comparison test. They concluded that Biodentine™ showed better marginal adaptation than commonly used root end filling materials.[38]
2. **Nanjappa et al (2015)** compared the sealing ability of mineral trioxide aggregate (MTA), Biodentine™, and Chitra-calcium phosphate cement (CPC) when used as root-end filling, and even evaluated under confocal laser scanning microscope using Rhodamine B dye and also evaluated effect of ultrasonic retro tip and by using an

erbium:yttrium aluminium garnet (Er:YAG) laser on the integrity of three different root-end filling materials. In this study,80 extracted teeth were instrumented and obturated with gutta -percha , tooth was resected at apical 3 mm and root-end preparation was made in depth of 3 mm using ultrasonic tip (n = 30) and Er:YAG laser (n = 30). MTA, Biodentine™, and Chitra-CPC were restored at each cavity. The samples were then coated with varnish and immersed in Rhodamine B dye for 24 h after drying . Under confocal laser scanning microscope the teeth were then rinsed, sectioned longitudinally, and observed .They concluded that root-end cavities were prepared with Er:YAG laser and restored with Biodentine™ showed superior sealing ability compared to those with ultrasonics.[39]

Bacteria with CLSM

1. **Abdul aziz et al (2012)** assessed disinfection of root end cavity through penetration of *Enterococcus faecalis* into the dentin of the apical 3 mm and its viability after the application of either chlorhexidine or laser to root-end cavities. In this study, 60 single-rooted teeth were taken, and chemomechanically prepared. In the first part, cementum was removed semicircumferentially from 21 roots, , and using 17% EDTA/cetrimide , the smear layer was removed from 15 roots and those teeth were inoculated and incubated with *E. faecalis* for 10 days, rinsed, and live/dead stained and bacterial penetration was assessed by confocal laser scanning microscopy (CLSM). In the second part, root ends were resected from remaining 39 teeth and cavities were ultrasonically prepared. The *E. faecalis* inoculated roots were grouped under (1) irrigated with 0.2 % chlorhexidine, (2) irradiated with a laser for 20 seconds at 1.5 W, or (3) received no treatment. Roots were examined for live/dead stained,

sectioned, and examined by Confocal Laser Scanning Microscope. Statistical Analysis were done using the Mann-Whitney U test. As results showed that in the apical 3 mm *E. faecalis* invaded the entire width of dentin, irrespective of the smear layer and/or cementum. Chlorhexidine was considered as more effective than laser in disinfecting root-end cavities.[40]

2. **Tsesis et al (2017)** evaluated colonization of *Enterococcus faecalis* at the apical part of root canals following root-end resection under confocal laser scanning microscopy (CLSM). 55 extracted single rooted human teeth were resected at apical third of 3 mm and retrograde cavities were prepared and filled with mineral trioxide aggregate (MTA), intermediate restorative material (IRM), or Biodentine™ (n = 10 each); 25 teeth served as controls. The roots were placed in apparatus, sterilized, and coronally filled with *E. faecalis* bacterial suspension for 21 days. The apical 3-mm segments were cut to get two slabs (coronal and apical). The viability of the bacteria were checked using LIVE/DEAD BacLight Bacterial stain Viability Kit and evaluated using CLSM. They concluded that the mean and maximal depths of bacterial colonization into the dentinal tubules were 755 μm and 1643 μm, respectively, with no differences between the root-end filling materials ($p > 0.05$). More live bacteria were found in the MTA group when compared with IRM and Biodentine™.[13]

MATERIALS AND METHODS

ARMAMENTARIUM USED

1. Extracted single rooted teeth

2. 0.3% thymol solution

3. Diamond disc and mandrel

4. Airotor Handpiece(NSK)

5. Micromotor handpiece (NSK)

6. K files (VDW, Germany)

7. Saline

8. 5.25% sodium hypochlorite.

9. Disposable syringe (Dispovan)

10. X-smart plus (Dentsply mallifer)

11. Protaper rotary files (Dentsply mallifer)

12. Protaper Gutta percha F3 size (Dentsply mallifer)

13.AH PLUS Sealer

14.RC HELP (Prime dental products)

15. Biodentine™ (Septodont, St. Maurdes Fossıs, France)

16.MTA Plus™ (Prevest denpro Limited)

17. Brasseler USA® *EndoSequence® Root Repair Material*™ (ERRM)

18.ULTRASONIC UNIT (Satlec)

19.S12 90 Retrotip

20. Eppendorf microtubes

21.Serum vials

22.Parafilm

23.Cyanoacrylate

24.*Enterococcus Faecalis*(ATCC 29212)

25.Brain Heart Infusion Broath

26.Laminar flow chamber

27.Micropipette

28. Self cure acrylic repair material

29.Microtome

INCLUSION CRITERIA

- Single rooted teeth
- Teeth with single root canal

EXCLUSION CRITERIA

- Teeth with multiple canals.
- Teeth with oval or ribbon shaped canals.
- Teeth with any anomalies like taurodontism
- Teeth with root resorption.

100 maxillary single rooted teeth were immersed in 5.25% NaOCI (sodium hypochlorite) to remove periodontal ligament remnants for 5 minutes and were stored in PBS (phosphate buffer solution) to prevent dehydration and the samples were autoclaved at 121°C for 15 min in 15 lb pressure. Decoronation of all tooth samples were done with 0.3 mm diamond disc for the standardization of working length of 16 mm. Radiographs were taken to confirm the working length with #10 k file and presence of only one root canal.

TOOTH SAMPLE PREPARATION

The chemomechanical preparation of root canals were done in crown down technique by using ProTaper rotary Ni-Ti instruments (Dentsply Maillefer, Ballaigues, Switzerland) up to a size of F3 with electrical endodontic handpiece (X-smart plus , Densply-Maillefer, Ballaigues, Switzerland) at 250 rpm with copious irrigation of 5 mL of freshly prepared solution of 2.5% NaOCl to remove biofilm, and 15% EDTA (Glyde; Dentsply Maillefer) for smear layer removal , followed by 2.5% NaOCl respectively in all the samples. Final irrigation was done with saline and the canals were dried with sterile paper points. Protaper F3 size Guttapercha and AH PLUS sealer were used for single cone obturation technique. The teeth were stored at 37°C ± 1°C and 100% relative humidity for 2 days.

The root resection was done in the apex at 3 mm perpendicularly to the long axis of the tooth with 0.3-mm diamond disc. The root end cavity was prepared by using ultrasonic retrotips (S1290, Gnatus Equipamento Medico-Odontologicos, Ribeirao Preto, SP, Brazil) about 3 mm depth. The teeth were randomly divided into 3 experimental groups and 2 control groups (positive and negative).

Group 1 (N= 20) – Retrograde cavities were filled using Biodentine™ (Septodont, France).

Group 2 (N=20) –Retrograde cavities were filled with Brasseler USA® *EndoSequence® Root Repair Material*™ (ERRM) paste

Group 3 (N=20) – Retrograde cavities were filled with MTA Plus™ (Prevest denpro Limited) with MTA carrier and condensed by plugger. Materials were mixed according to manufacture's instructions.

Group 4 (N = 20) (positive control) - Retrograde cavities were left without retrograde filling.

Group 5 (N = 5) (negative control) - Retrograde cavities were filled with sticky wax and the entire root surface including the apical portion was covered with two layers of nail varnish.

All the experimental groups were kept in an incubator at 37 °C in 100 % humidity for 1 week.

In all other groups, two layers of nail varnish was applied on the entire surface of the sample except in the apical portion.

DUAL CHAMBER METHOD

Following the design of apparatus as described by siqueria et al , dual chamber apparatus was prepared . [41] The tip of the eppendorf microtubes were cut and the samples were inserted into it upto 4 mm from the apex. The cyanoacrylate was applied to seal the interface between tooth and the tube and parafilm was wrapped around it. Serum vials (20 mL) with rubber stops act as second chamber. Both of these apparatus were sterilised in ethylene oxide (EtO Chamber) for 6 hrs at 45° C.

BACTERIAL INOCULATION

Enterococcus faecalis (ATCC 29212) in blood agar plate was bought from Bioline Laboratories .Then the bacterial culture was prepared in Brain Heart infusion suspension and incubated at 37°C for 24 hours. According to the MacFarland scale , bacterial cells were resuspended to 3×10^{8} colony-forming units (CFU)/mL. The 5 µl of bacterial culture was inoculated in reservoir of each tooth specimen kept inside the microtubes using sterile

micropipettes every alternate days. The root-ends were immersed in sterile Brain Heart Infusion broath . The apparatus was incubated at 37°C for 45 days and checked daily for turbidity in the BHI broth. Species-specific polymerase chain reaction (PCR) was performed to confirm the identification of *Enterococcus faecalis* in samples . The density of the turbidity occurred was measured in ELISA READER at the end of 45^{th} day in all specimens .The mean and standard deviation was calculated and results were compared using ANOVA and TUKEY HSD with a significant difference of $p<0.05$ value.

CONFOCAL LASER SCANNING MICROSCOPIC EVALUATION

A cylindrical block about 50 mm was made in self cure acrylic repair material for each samples and tooth were embedded in it exposing the apical portion. The apical portion were cut in 20 µm length perpendicular to the long axis of the cylindrical block using microtome.

All the tooth samples were stained with Fluoroscent diacetate and Propiodium Iodide dye to check the LIVE/DEAD bacteria and also to quantify the *Enterococcus Faecalis* in Confocal Laser Scanning Microscope (ZEISS LSM 800) under 20X and 10X magnification. Statistical analysis were done with ANOVA and TUKEY test, the significant difference value were set at $p<0.05$.

ARMAMENTARIUM FOR BIOMECHANIAL PREPARATION

Figure 1: Xmart plus (Dentsply), Irrigant solution, K files, Protaper files (SX,S1,S2,F1,F2,F3), Gutta percha cones, (F3), AH Plus sealer

ARMAMENTARIUM USED FOR RETROGRADE PREPARATION AND MATERIALS

Figure 2: Ultrasonic Unit (Satlec) , S12 90 Retrotip , Biodentine™, MTA Plus™, *EndoSequence® Root Repair Material™*

Fig 3 : Teeth samples

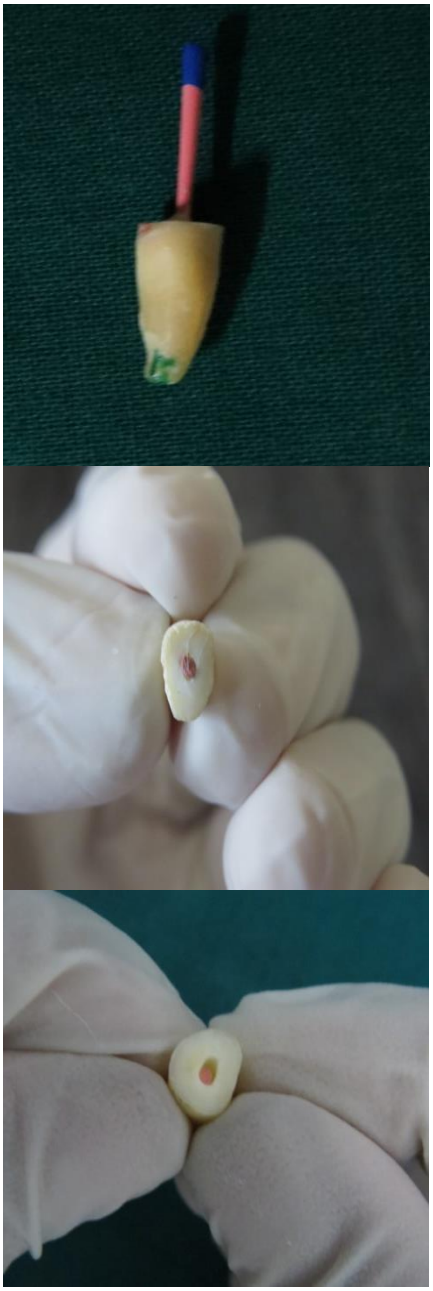


Fig 4 :Tooth after obturation and 3mm removed from coronal seal to act as reservoir

Fig 5 : Root end sectioned at 3 mm from the apex after obturation

Fig 6 : Retrograde cavity preparation with ultrasonics retrotips (S1290, Gnatus Equipamento Medico-Odontologicos, Ribeirao Preto, SP, Brazil)

Fig 7 : Retrogarde filling with Biodentine™

Fig 8 : Retrogarde filling with ERRM

Fig 9 : Retrogarde filling with MTA Plus™

ARMAMENTARIUM FOR DUAL CHAMBER MODEL

Fig 10 : . Eppendorf microtubes , Serum vials , Parafilm , Cyanoacrylate

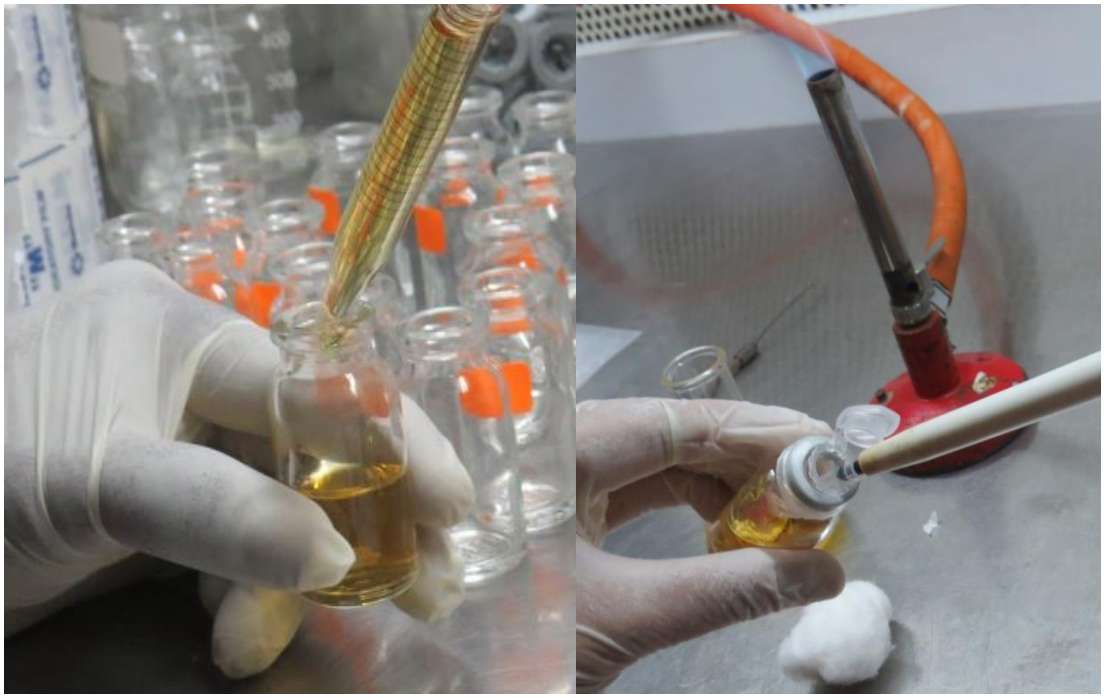


Fig 11 : Inoculation of *Enterococccus faecalis*

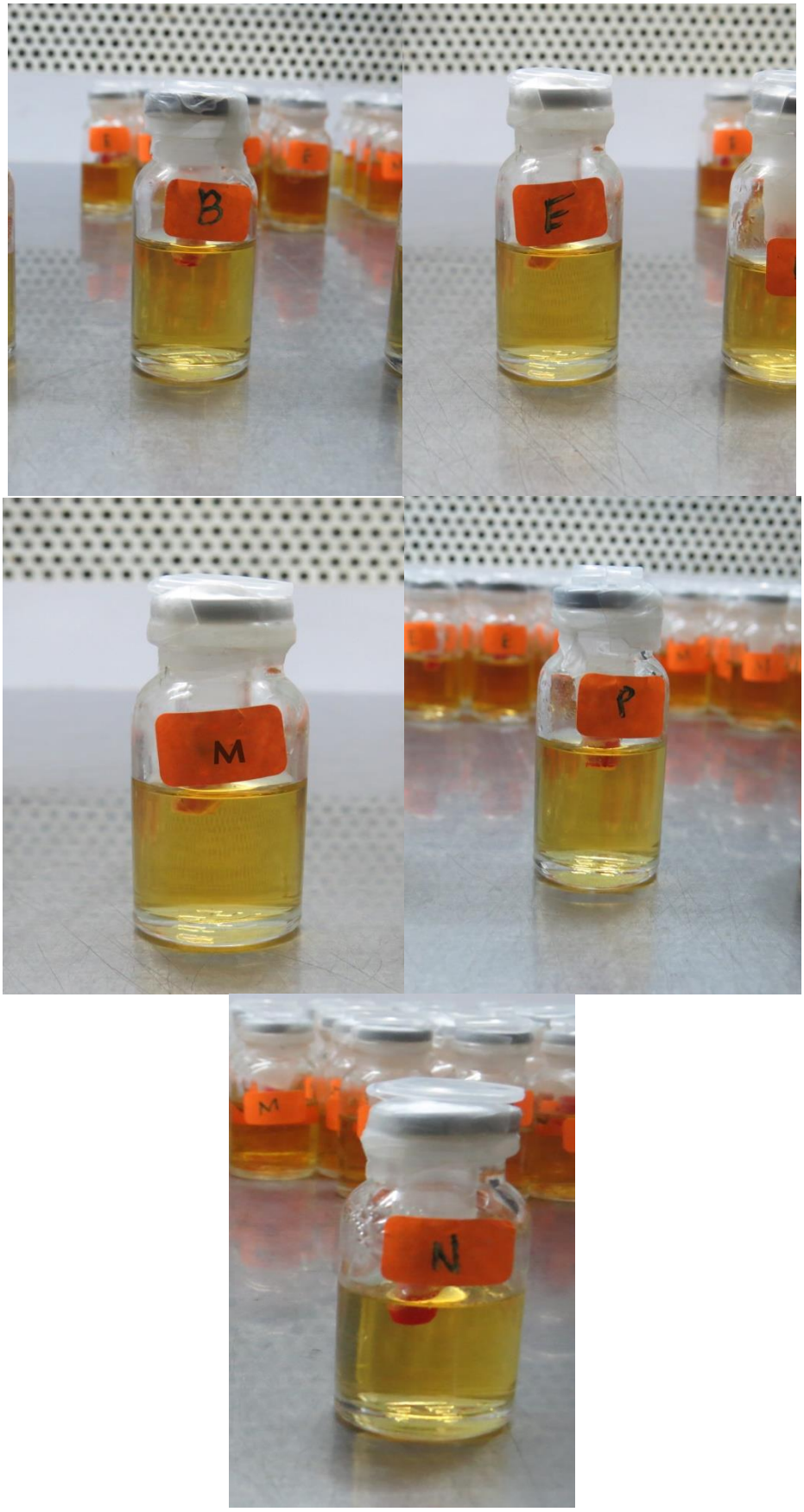


Fig 12: Dual chamber model

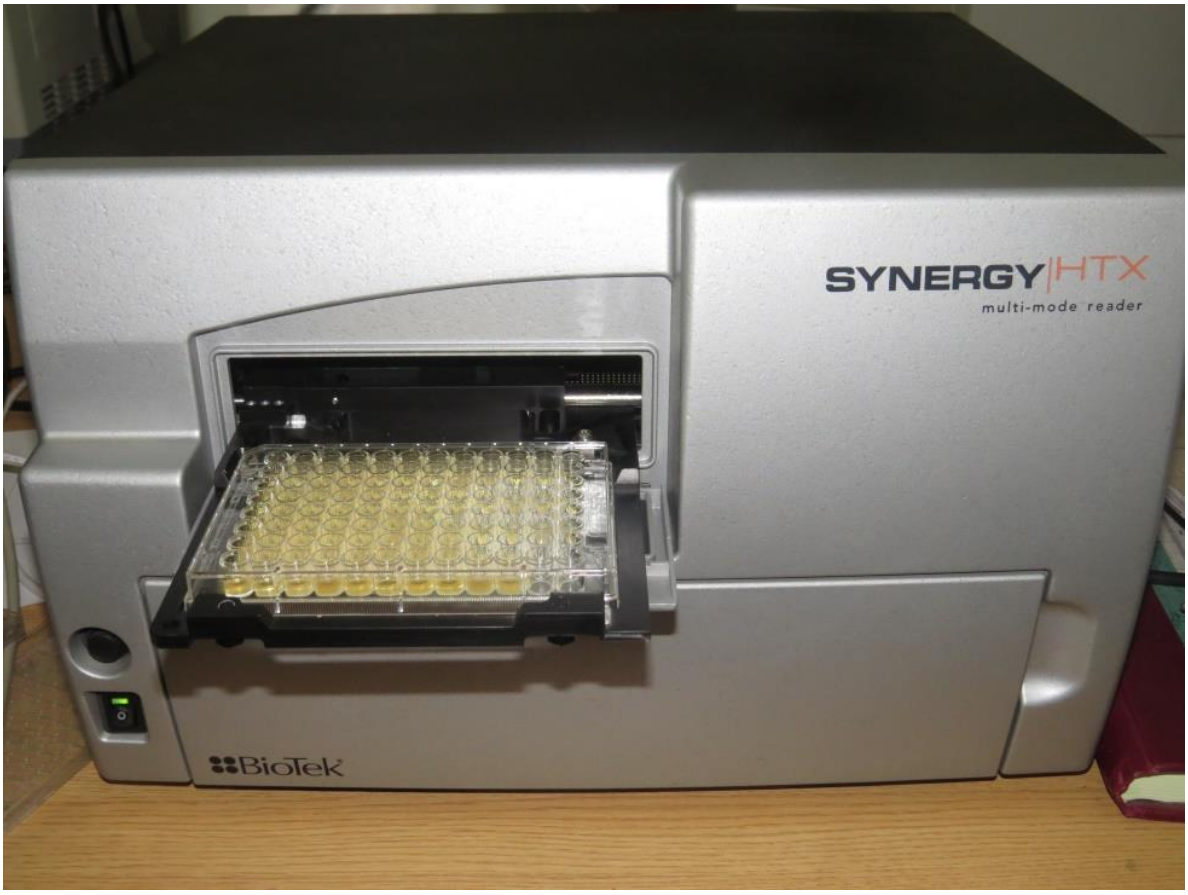


Fig 13 : ELISA Reader

Fig 14 : Acrylic cylindrical blocks prepared for tooth sectioning to examine under CLSM

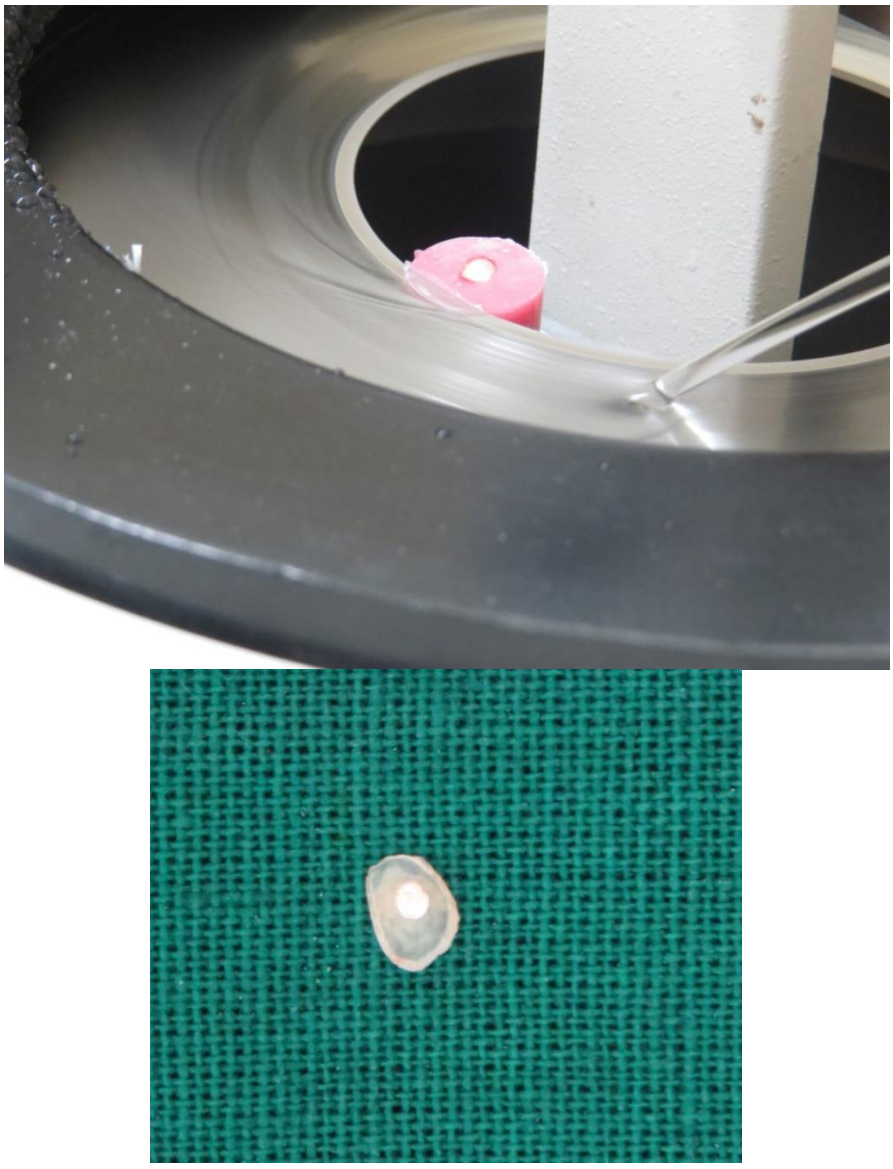


Fig 15 : Tooth Sectioned In Microtome

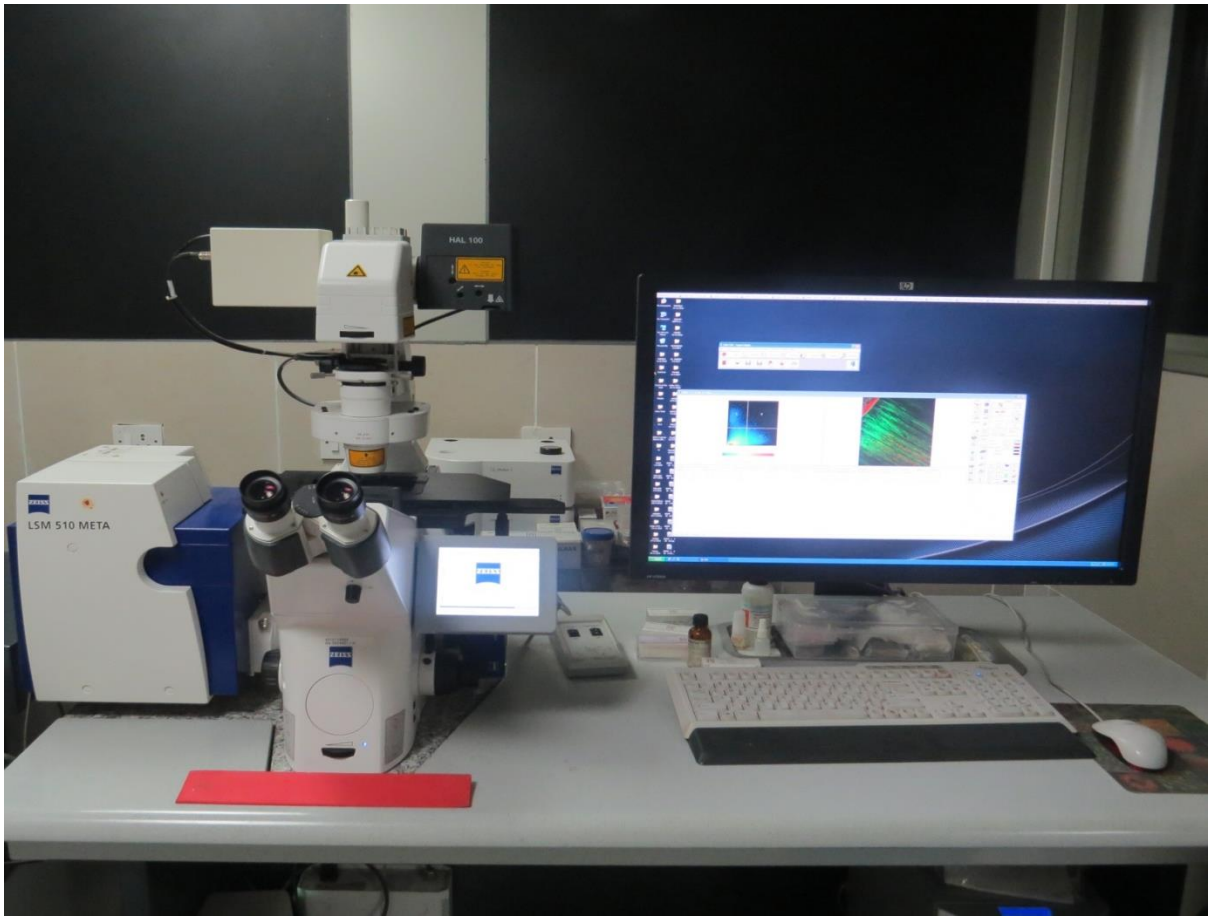


Fig 16 : Confocal Laser Scanning Microscope

RESULTS

SL NO.	BIODENTINE™			ERRM			MTA PLUS™			POSITIVE		
1	33	33	32	56	62	59	53	54	56	98	89	98
2	93	25	96	44	33	34	41	25	40	96	98	90
3	92	29	57	23	32	43	23	18	99	98	89	99
4	53	52	68	76	54	23	62	63	84	98	97	95
5	58	51	50	12	65	32	69	60	68	94	99	96
6	48	87	96	41	54	56	30	84	42	98	92	96
7	54	92	59	74	32	34	68	57	75	97	100	97
8	41	44	52	25	26	24	62	99	68	96	94	98
9	49	50	50	34	78	32	27	51	23	93	92	93
10	62	99	40	43	34	21	89	55	55	92	93	94
11	34	32	50	26	24	23	35	68	49	97	98	99
12	48	51	33	76	87	54	39	94	60	93	91	98
13	33	35	37	67	89	87	17	17	42	98	92	94
14	38	38	41	23	35	12	11	13	77	94	95	96
15	46	46	43	28	34	22	53	52	55	98	99	98
16	42	44	44	21	56	34	37	67	11	99	98	99
17	69	65	57	23	21	75	11	10	13	100	98	97
18	46	48	40	34	20	32	54	51	95	96	97	98
19	34	30	52	14	23	15	84	90	41	98	99	99
20	52	50	43	26	23	17	48	61	32	99	99	97

Table 1 : Measurement of turbidity

Table – 1 represents the triplicate values of each group measured in ELISA READER for turbidity change

SL NO.	BIODENTINE™	ERRM	MTA PLUS™
1	240.34	227.37	277.74
2	238.38	226.30	234.37
3	242.95	215.56	236.04
4	303.50	243.95	248.67
5	209.08	209.16	347.04
6	234.62	211.41	288.73
7	237.28	206.65	267.97
8	245.65	256.9	282.76
9	248.05	217.67	289.44
10	211	237.19	311.16
11	244.68	208.34	297.03
12	237.02	212.74	255.98
13	256.86	195.32	245.7
14	234.42	137.67	284.88
15	218.24	143.54	317.11
16	211.54	167.78	225.7
17	246.52	134.54	233.16
18	231.48	136	295.91
19	255.07	154.9	266.94
20	278.40	132.56	321.01

Table 2 :Depth of penetration of *E.Faecalis* through retrograde material (µm)

Table -2 represents the values for depth of penetration of *Enterococccus faecalis* into the dentinal tubules

PCR

```
+1    GAACGCTGGC GGCGTGCCTA ATACATGCAA GTCGAACGCT TCTTTCCTCC  50
51    CCGAGTGCTT GCACTCAATT GGAAAGAGGA GTGGCGGACG GGTGAGTAAC  100
101   ACGTGGGTAA CCTACCCATC AGAGGGGGAT AACACTTGGA AACAGGTGCT  150
151   AATACCGCAT AACAGTTTAT GCCGCATGGC ATAAGAGTGA AAGGCGCTTT  200
201   CGGGTGTCGC TGATGGATGG ACCCGCGGTG CATTAGCTAG TTGGTGAGGT  250
251   AACGGCTCAC CAAGGCCACG ATGCATAGCC GACCTGAGAG GGTGATCGGC  300
301   CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG CAGCAGTAGG  350
351   GAATCTTCGG CAATGGACGA AAGTCTGACC GAGCAACGCC GCGTGAGTGA  400
401   AGAAGGTTTT CGGATCGTAA AACTCTGTTG TTAGAGAAGA ACAAGGACGT  450
451   TAGTAACTGA ACGTCCCCTG ACGGTATCTA ACCAGAAAGC CACGGCTAAC  500
501   TACGTGCCAG CAGCCGCGGT AATACGTAGG TGGCAAGCGT TGTCCGGATT  550
551   TATTGGGCGT AAAGCGAGCG CAGGCGGTTT CTTAAGTCTG ATGTGAAAGC  600
601   CCCCGGCTCA ACCGGGGAGG GTCATTGGAA ACTGGGAGAC TTGAGTGCAG  650
651   AAGAGGAGAG TGGAATTCCA TGTGTAGCGG TGAAATGCGT AGATATATGG  700
701   AGGAACACCA GTGGCGAAGG CGGCTCTCTG GTCTGTAACT GACGCTGAGG  750
751   CTCGAAAGCG TGGGGAGCAA ACAGGATTAG ATACCCTGGT AGTCCACGCC  800
801   GTAAACGATG AGTGCTAAGT GTTGGAGGGT TTCCGCCCTT CAGTGCTGCA  850
851   GCAAACGCAT TAAGCACTCC GCCTGGGGAG TACGACCGCA AGGTTGAAAC  900
901   TCAAAGGAAT TGACGGGGGC CCGCACAAGC GGTGGAGCAT GTGGTTTAAT  950
951   TCGAAGCAAC GCGAAGAACC TTACCAGGTC TTGACATCCT TTGACCACTC  1000
1001  TAGAGATAGA GCTTTCCCTT CGGGGACAAA GTGACAGGTG GTGCATGGTT  1050
1051  GTCGTCAGCT CGTGTCGTGA GATGTTGGGT TAAGTCCCGC AACGAGCGCA  1100
1101  ACCCTTATTG TTAGTTGCCA TCATTTAGTT GGGCACTCTA GCGAGACTGC  1150
1151  CGGTGACAAA CCGGAGGAAG GTGGGGATGA CGTCAAATCA TCATGCCCCT  1200
1201  TATGACCTGG GCTACACACG TGCTACAATG GGAAGTACAA CGAGTCGCTA  1250
1251  GACCGCGAGG TCATGCAAAT CTCTTAAAGC TTCTCTCAGT TCGGATTGCA  1300
1301  GGCTGCAACT CGCCTGCATG AAGCCGGAAT CGCTAGTAAT CGCGGATCAC  1350
1351  ACGCCCGGTG AAGAAAATTT TCCCGGGCCC TTGTACACCC GCCCGTCACA  1400
1401  CCACGAGAGT TTGTAACACC CGAAGTCGGT GAGGTAACCT TTTTGGAGCC  1450
1451  AGCCGCCTAA GGTGGGATAG ATGATTGGGG TGAAGTCGTA A 149
```

PCR

RDP: Release 11

SeqMatch :: Detail Hierarchy

[new match | summary | detail | help]

[redacted]

Query Sequence: seqmatch_seq, 1419 unique oligos

Match hit format:
short ID, orientation, similarity score, S_ab score, unique common oligomers and sequence full name. More help is available.

Lineage:

- rootrank Root (0/1/12736) (selected/match/total RDP sequences)
- domain Bacteria (0/1/12227)
- phylum Firmicutes (0/1/2438)
- class Bacilli (0/1/1569)
- order Lactobacillales (0/1/504)
- family Enterococcaceae (0/1/67)
- genus Enterococcus (0/1/50)
  - S000427506 not_calculated 0.950 1434 Enterococcus faecalis (T); JCM 5803; AB012212

Data Set Options:

	Type ● Non Type ●	Both
Source	Uncultured ● Isolates ●	Both
	≥1200 ● <1200 ● good ●	Both
Quality	Suspect ●	Both

KNN matches: [redacted]

Strain: Type strain information is provided by bacterial taxonomy. *Hint:* Type strains link taxonomy with phylogeny. Include type strain sequences in your analysis to provide documented landmarks.

Source: View only environmental (uncultured) sequences, only sequences from individual isolates, or both. Source classification is based on sequence annotation and the NCBI taxonomy.

Size: View only near-full-length sequences (≥1200 bases), short partials, or both.

Quality: View only good quality sequences, suspect quality sequences, or both. Sequences were flagged (*) as suspect quality. [more quality detail]

KNN matches: Number of matches displayed per sequence, also number used to classify queries by unanimous vote.

Questions/comments: rdpstaff@msu.edu

Fig – 17 and Fig – 18 shows the Group 1 (Biodentine™) for the depth of penetration of *Enterococcus faecalis* into the dentinal tubules under 20x and 10x magnification

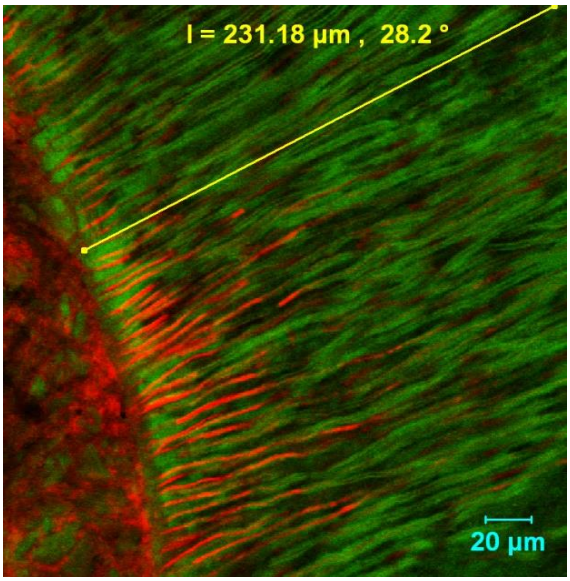


Fig 17 : Depth of penetration of *E. faecalis* in BIODENTINE™ under 20x magnification

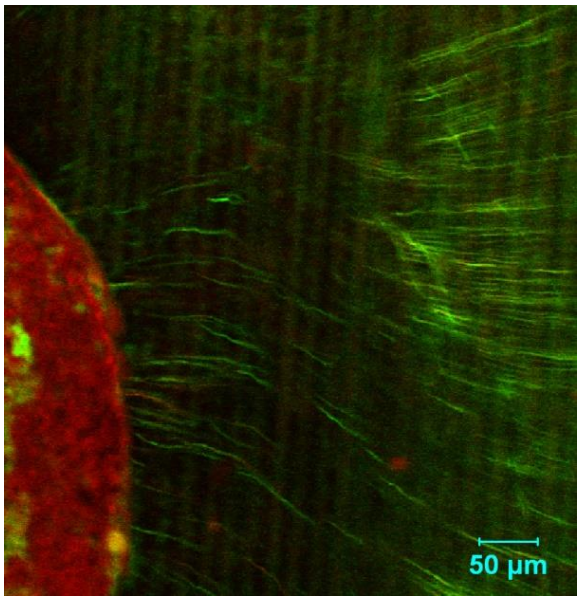


Fig 18 : Depth of penetration of *E. faecalis* in BIODENTINE™ under 10x magnification

Fig – 19 and Fig - 20 shows the Group 2 (*EndoSequence® Root Repair Material™*) for the depth of penetration of *Enterococcus faecalis* into the dentinal tubules under 20x and 10x magnification

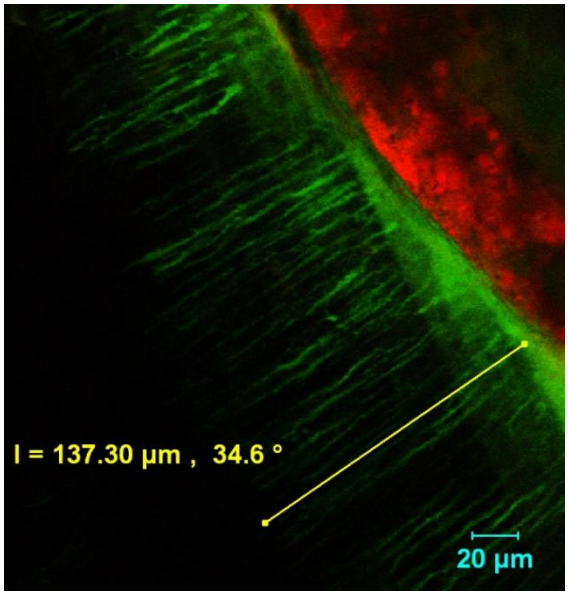


Fig 19 : Depth of penetration of *E. faecalis* in ERRM under 20x magnification

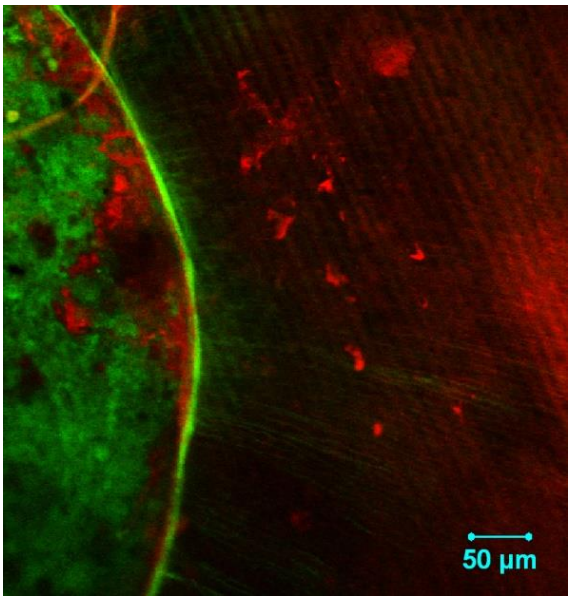


Fig 20 : Depth of penetration of *E. faecalis* in ERRM under 10x magnification

Fig- 21 and Fig- 22 shows the Group 3 (MTA Plus^{TM}) for the depth of penetration of *Enterococcus faecalis* into the dentinal tubules under 20x and 10x magnification

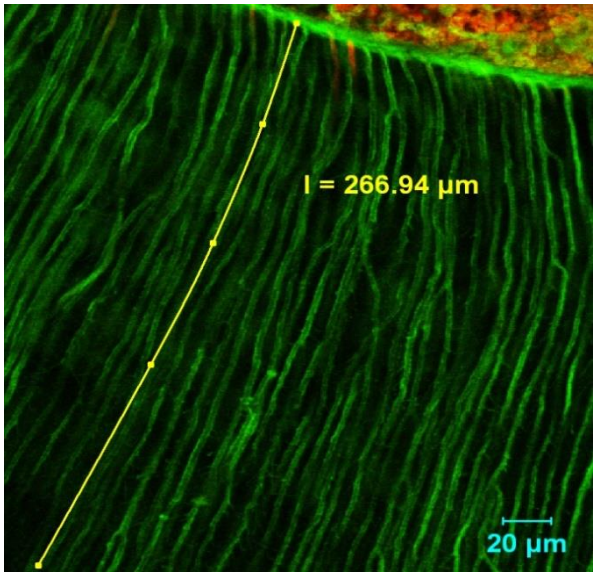


Fig 21 : Depth of penetration of *E. faecalis* in MTA plus^{TM} under 20x magnification

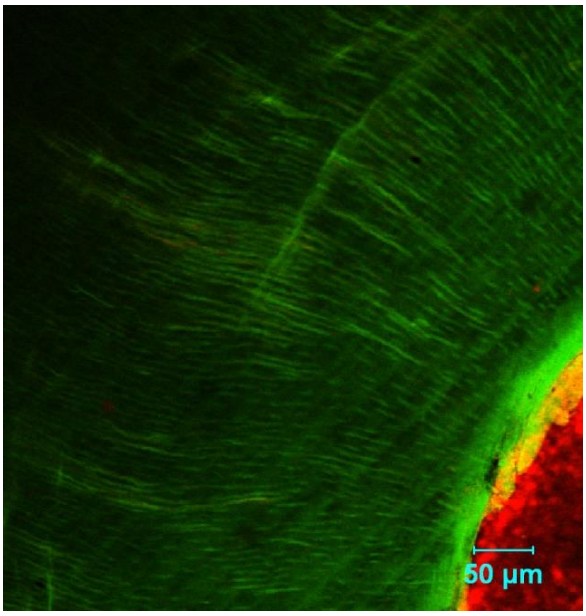


Fig 22 : Depth of penetration of *E. faecalis* in MTA plus^{TM} under 10x magnification

STATISTICAL ANALYSIS

Turbidity Average

Group	N	Mean	Std. Deviation	Std. Error
Biodentine™	20	51.0333	13.28055	2.96962
ERRM	20	39.6167	16.88706	3.77606
MTA Plus™	20	51.4500	17.50732	3.91476
Positive Control	20	96.3833	1.59045	.35564
Total	80	59.6208	25.77511	2.88175

Table 3:Mean, Standard deviation for turbidity measurement

ANOVA

Turbidity Average

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	37843.115	3	12614.372	65.480	.000
Within Groups	14641.050	76	192.645		
Total	52484.165	79			

Table 4: ANOVA for turbidity measurement

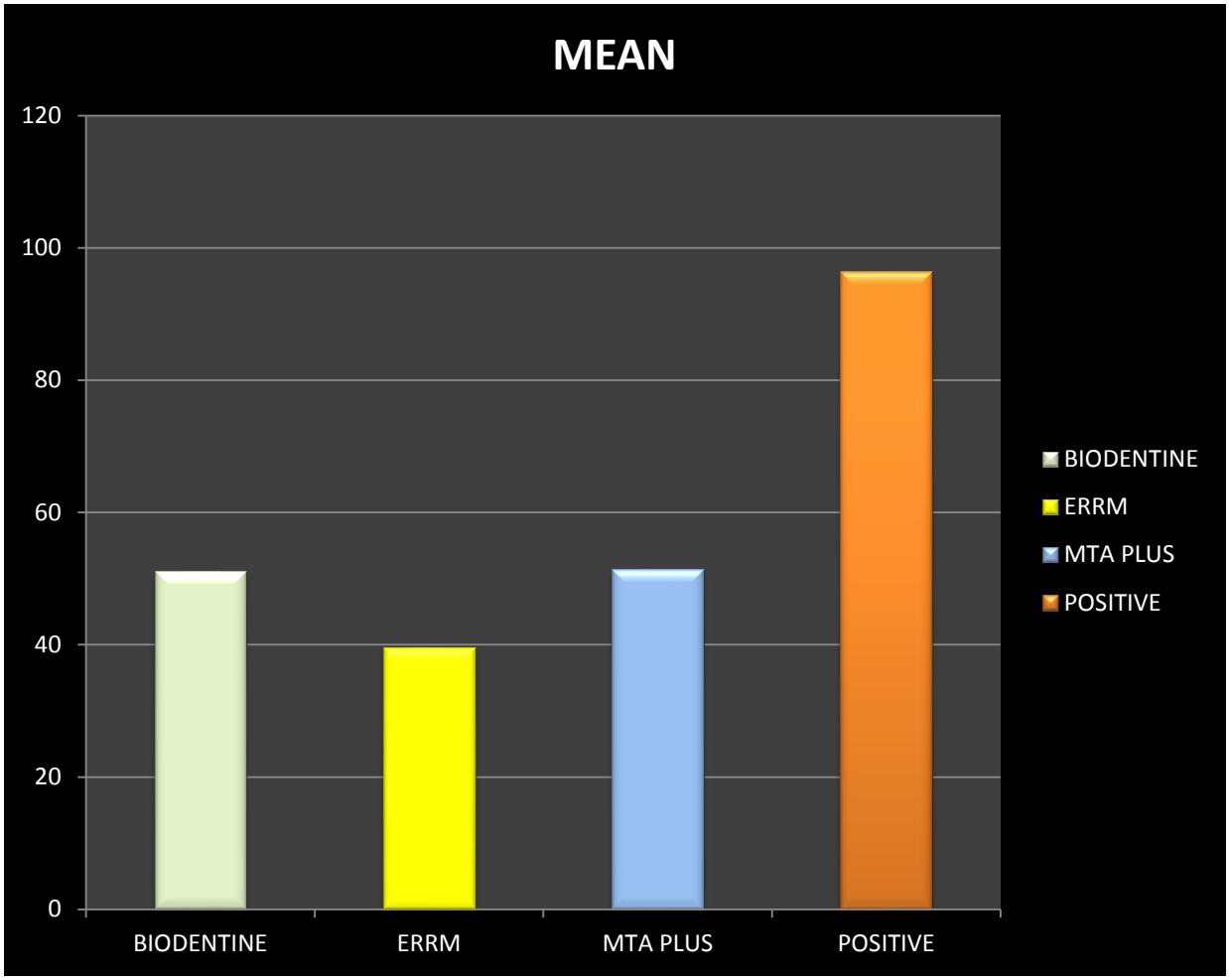
MULTIPLE COMPARISONS

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
Biodentine™	ERRM	11.41667	4.38914	.053
	MTA Plus™	-.41667	4.38914	1.000
	Positive Control	-45.35000*	4.38914	**.000**
ERRM	Biodentine™	-11.41667	4.38914	.053
	MTA Plus™	-11.83333*	4.38914	**.042**
	Positive Control	-56.76667*	4.38914	**.000**
MTA Plus™	Biodentine™	.41667	4.38914	1.000
	ERRM	11.83333*	4.38914	**.042**
	Positive Control	-44.93333*	4.38914	**.000**
Positive Control	Biodentine™	45.35000*	4.38914	**.000**
	ERRM	56.76667*	4.38914	**.000**
	MTA Plus™	44.93333*	4.38914	**.000**

*. The mean difference is significant at the 0.05 level.

Table 5: TUKEY HSD for turbidity measurement



Graph 1 : Turbidity measurement

Descriptives

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	68719.776	2	34359.888	31.609	.000
Within Groups	61960.662	57	1087.029		
Total	130680.438	59			

Table 6:Mean, Standard deviation of Depth of penetration of *Enterococcus faecalis* into dentinal tubules

Group	N	Mean	Std. Deviation	Std. Error
Biodentine™	20	241.25	22.22939	4.97064
ERRM	20	193.78	40.62616	9.08429
MTA Plus™	20	276.37	33.41342	7.47147
Total	60	237.13	47.06297	6.07580

Table 7:ANOVA for Depth of penetration *of Enterococcus faecalis* into dentinal tubules

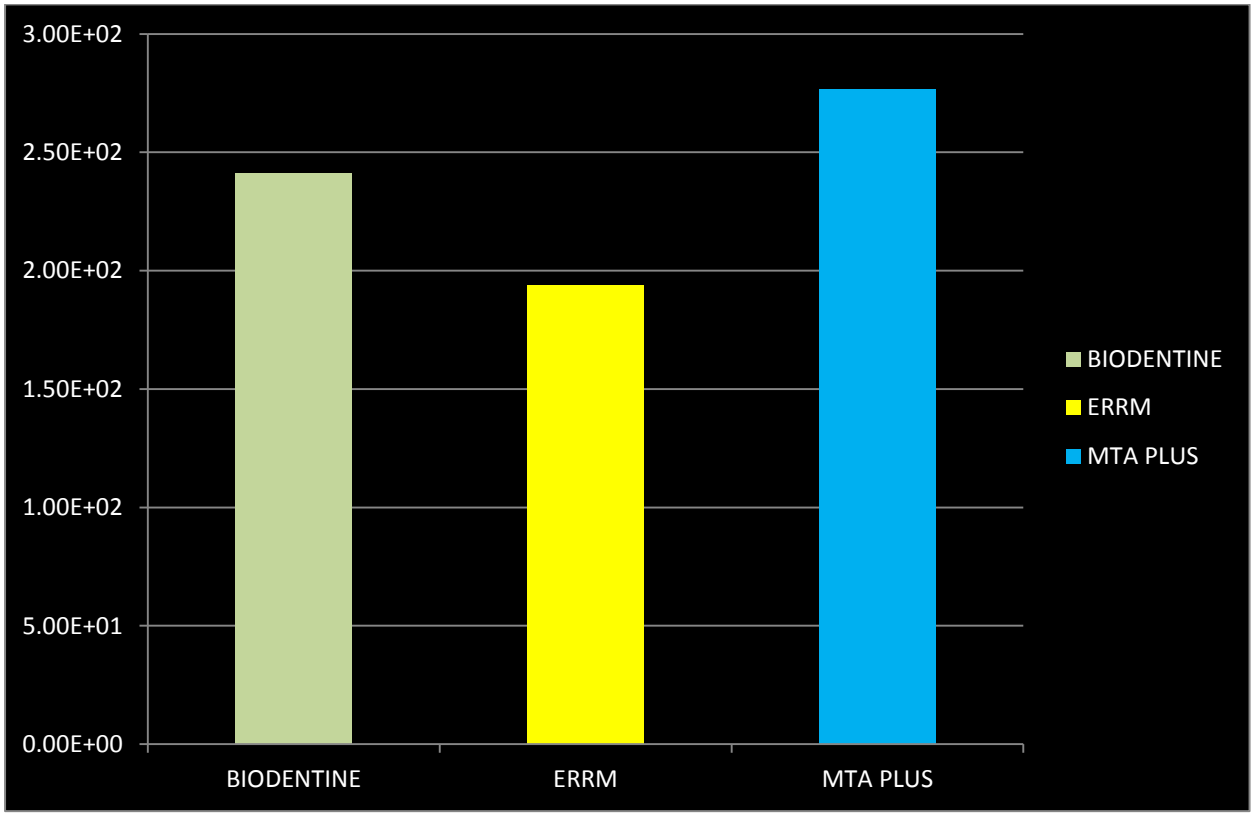
Depth

Tukey HSD

		Mean Difference (I-J)	Std. Error	Sig.
Biodentine™	ERRM	47.47650*	10.42607	.000
	MTA Plus™	-35.11300*	10.42607	.004
ERRM	Biodentine™	-47.47650*	10.42607	.000
	MTA Plus™	-82.58950*	10.42607	.000
MTA Plus™	Biodentine™	35.11300*	10.42607	.004
	ERRM	82.58950*	10.42607	.000

*. The mean difference is significant at the 0.05 level.

Table 8: TUKEY HSD for Depth of penetration of *Enterococcus faecalis* into dentinal tubules



Graph 2 : Depth of penetration of *Enterococcus faecalis* in µm

The mean and standard deviation for turbidity change are presented in Table 3. **ANOVA** and **TUKEY HSD** values for turbidity change are presented in Table 4 and Table 5. Graph 1 represents the line diagram of mean distribution of all the groups.

Turbidity measurement

The results of present study showed that *EndoSequence® Root Repair Material™* (ERRM) had statistically significant difference in turbidity change when compared MTA Plus™ and positive control. Biodentine™ and ERRM showed only less statistically significant difference. When comparing between groups MTA Plus™ and Biodentine™ showed no statistically significant difference , In between intergroups , there is statistically significant difference. (P value is < 0.05; ANOVA-Tukey HSD).

The mean and standard deviation for depth of penetration of *Enterococcus faecalis* into the dentinal tubules are presented in Table 6. **ANOVA** and **TUKEY HSD** values for depth of penetration are presented in Table 7 and Table 8. **Fig 17 to 22** represents Confocal laser scanning microscopic images at 20x and 10x of all experimental groups for the Depth of penetration of *Enterococcus faecalis*..Graph 2 represents the mean distribution of bacterial penetration in CLSM of three experimental groups.

Depth of penetration

The results showed that there is statistically significant difference in depth of penetration of *Enterococcus faecalis* into the dentinal tubules between ERRM, Biodentine™ and MTA Plus™ (P value is < 0.05; ANOVA-Tukey HSD). The microbial leakage of *Enterococcus faecalis* through retrograde filling materials showed different results in turbidity change and in depth of penetration. *EndoSequence® Root Repair Material™* (ERRM) showed better results in bacterial leakage and also in depth of penetration into the dentinal tubules . Biodentine™ and MTA Plus™ showed similar results with more leakage in

dual chamber whereas bacterial penetration into the dentinal tubules in CLSM , there is statistical significant difference between Biodentine™ and MTA Plus ™ .

DISCUSSION

DISCUSSION

The apical seal plays an important role in the success of surgical endodontics. The characteristics which make an ideal root end filling material are adequate adhesion to the root end cavity walls, prevention of microleakage to the periradicular tissues, biocompatibility, insoluble in tissue fluids, dimensionally stable.[2] Following the periapical surgery, penetration of bacteria into the periapical tissues may occur in some cases in such a way that bacterial colonization occurs, leads to reinfection of the dentin. Through cell division and availability of nutrients, bacterial colonization becomes doubled and the dentinal tubules may eventually become a safe haven for bacteria. Thus the adhesion between filling dentin interphase becomes important.

In most of the previous studies dye leakage were used, due to their low molecular weight , accessible to the area where the proteins and bacteria cannot penetrate , however as they lack uniformity in the marginal penetration and in correlating the clinical condition , most of the studies have proven that bacterial leakage studies are better in evaluating the sealing ability when compared with other studies like microbiological, histological, and microscopic techniques. Hence in this study, bacterial leakage analysis was done with *Enterococcus faecalis*, since it is the most predominant bacteria in chronic apical periodontitis of failed root canal therapies.[27]

In our study , the results showed that ERRM provides a better apical seal as root end filling than Biodentine™ and MTA Plus™. As mentioned in the previous studies by Antunes and Nair et al, ERRM has the similar sealing ability compared to MTA with 92 % of high healing rates [35] Even though MTA is the gold standard for root end filling material it has some disadvantages like technique sensitive, long setting time, low washout resistance, difficult handling properties and also cause tooth discoloration. To overcome these limitations , ERRM has certain advantages like does not cause tooth discoloration , forms

ultimately hydroxyapatite in contact with moisture , better bond between with dentin , available in premixed intermediate condition which aids in ease of handling , alkaline pH which exhibits antibacterial activities against *Enterococcus faecalis* .[42]

Biodentine ™ is a bioactive dentine substitute , high specific surface of particle size and calcium chloride in liquid accelerates the system which favours fast setting time [16]. The tricalcium silicates are free of metallic impurities which avoids discoloration and on hydration it forms calcium silicate hydrate gel and calcium hydroxide which favours expansion of the material . It also holds antibacterial properties due to its high pH and this alkalinity leads to the disinfection of adjacent hard and soft tissue structures.[13] It increases cell proliferation , biomineralisation , formation of tag like structures helps in maintaining its bond strength even in the presence of blood.[22]

MTA Plus™ consist of finer particle size than ordinary MTA which improves handling and ease of placement. The mixing of powder particles with gel adds on anti-washout property to the material. One of the drawback of MTA is tendency to washout during irrigation in the retrograde cavities before closure of periapical flap . Upon hydration, formation of calcium silicate hydrate occurs and in addition , to increase the flow characteristics and to modify the setting time , gel should be added more . Similar to our study as detailed by Kokate and Parwar (2012) and Ravichandra *et al*.(2014)[39], there were no significant differences between the sealing abilities of Biodentine™ and MTA . Seedat et al (2016) compared sealing ability of White ProRoot MTA, MTA Plus and Biodentine™, found there is no significant difference between the above calcium silicate cements.[25].

Various microscopic techniques to assess the sealing ability of root end filling materials available includes stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal laser scanning microscopy (CLSM).Among them CLSM is the traditional microbiological histological standard electron

microscopy, in which the live/dead staining methods are used which provides information about the vitality of bacteria in the infected dentinal tubules.[13]

The cytotoxicity of the root end filling material may cause the degeneration of the periapical tissues , also delays wound healing by affecting the periodontal ligament fibroblast (PDLF), where toxic elements from the material could leak into surrounding bony crypt. Regarding ERRM , the study conducted by Hernán et al [23] observed that in PDLF and in gingival fibroblast , MTA Angelus® and EndoSequence Root Repair Material Putty® were less cytotoxic compared with Super EBA® , IRM and Cavit in higher dilution.

The findings of this study doesnot correlates with the results as described by Shokouhinejad et al in which ERRM Paste and Putty showed similar marginal adaptation as compared with MTA.[18] whereas Nagesh et al showed less marginal gap when compared with MTA.[24]

A scenario which signifies clinical condition should have a dentin infection model . As the controversies in evaluating the actual routes of bacterial penetration and colonization ,there are certain limitations in the traditional two-chamber model of bacterial leakage like real routes of the bacterial colonization in the experimental groups, and the absence of proper histological controls. In the current study, the two-chamber model of bacterial leakage was used, in which positive and negative controls were used to confirm the adequacy of the experimental model. In the negative control group, no turbidity was observed, while in all the specimens of the positive control group turbidity were found thus ensuring the adequacy of the experimental model.[13] Apart from the turbidity, the depth of penetration of bacteria was measured in Confocal laser scanning microscope.

A hydration reaction is required during setting reaction to acquire the final properties of the material. However with adequate water the bioceramic materials expand and shrinkage occurs during dry environment . In normal clinical conditions , the root end filling

materials were not submerged in saline or water , but limited presence of body fluids in the periapex favours shrinkage .[44] In this study, instead of saline or water , root end filling materials were immersed in Brain Heart Infusion broath , where ERRM shower better results when compared with Biodentine™ and MTA Plus™ . Similar to this study, chen et al observed that ERRM has good sealing ability than MTA with significantly greater surface area covered by cementum-like tissue, PDL-like tissue, and bone adjacent to the resected root-end surfaces in a dog model and was detected by using CBCT and micro CT . [45]

In previous study it was found that the microstructural changes and cracks were observed at the root dentine to Biodentine interphase in dry environment which had the potential to allow the ingress and transmission of microorganisms. [43]

In this study, ERRM had significant difference when compared with Biodentine™ and MTA Plus™. The less leakage of bacteria was found in ERRM (39.6167), whereas in Biodentine™ (51.0333) and MTA Plus™ (51.4500) showed more leakage by analysing the turbidity . The results over confocal laser scanning microscope showed ERRM (193.78) had less penetration of bacteria when compared with Biodentine™ (241.25) and MTA plus™ (276.37) . Even though ERRM and biodentine™ form tag like structures with dentinal tubules, ERRM showed better results than biodentine™ and MTA Plus™ . The better sealing ability of ERRM might be due to the bioactivity of the calcium silicate cements which gained chemical bonding of hydroxyapatite [25] crystals to the radicular dentine.

The results of this study were histologically better validated by confocal laser scanning microscope. On evaluation , all the newer bioceramic materials showed significant difference in bacterial leakage and bacterial penetration into the dentinal tubules . Among the test materials ERRM showed better results than Biodentine™ and MTA Plus™.

SUMMARY

SUMMARY

The present study was conducted in the Department of Conservative Dentistry and Endodontics, KSR Institute of dental science and research which has been approved from instituitional review board. Hundred single rooted teeth were root canal treated, apically sectioned at 3mm and filled with 3 new bioceramic materials (n=20) such as *EndoSequence® Root Repair Material™*, Biodentine ™ and MTA Plus ™ . Dual chamber model was prepared and *Enterococcus faecalis* were inoculated, kept for 45 days. The bacterial leakages were confirmed with turbidity change through ELISA reader, to validate the results histologically specimens were checked under Confocal laser scanning microscope.

The findings of the present study is summarized as follows

1. There was a statistically significant difference in the bacterial leakage and bacterial penetration into the dentinal tubules between all the test materials.
2. *EndoSequence® Root Repair Material™* (ERRM) showed better results in both bacterial leakage and in bacterial penetration
3. There is no statistical difference between Biodentine™ and MTA Plus ™ in bacterial leakage and whereas in bacterial penetration there is statistical significant difference between Biodentine™ and MTA Plus™ .

CONCLUSION

CONCLUSION

The following inference has been derived from this study. *EndoSequence® Root Repair Material™* (ERRM) showed better sealing ability than Biodentine™ and MTA Plus™. In this study , Confocal laser scanning microscope has been used to confirm bacterial penetration histologically, to overcome the limitations of dual chamber method. Further invivo studies has to be done to prove the efficacy of *EndoSequence® Root Repair Material™* .

BIBILIOGRAPHY

BIBLIOGRAPHY

1. Carrotte P. Endodontics: Part 4. Morphology of the root canal system. Br Dent J 2004; 197: 289–367.
2. *Torabinejad M, Watson T, Pitt Ford T. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod 1993;19(12):591-595.*
3. *Haapasalo M, Shen Y. Current therapeutic options for endodontic biofilms. Endodontic Topics 2010;22(1):79-98.*
4. Tsesis I, Faivishevsky V, Kfir A, Rosen E. Outcome of surgical endodontic treatment performed by a modern technique: a meta-analysis of literature. J Endod 2009;35(11):1505-11.
5. Gartner AH, Doran SO. Advances in endodontic surgery. Dent. Clin. N. Amer. 1992; 36: 357-379.
6. Vasudev SK, Goel BR, Tyagi S. Root end filling materials-A review. Endodontology 2003;15:12-8.
7. Soundappan S, Sundaramurthy JL, Raghu S, Natanasabapathy V. Biodentine versus Mineral Trioxide Aggregate versus Intermediate Restorative Material for retrograde root end filling: An invitro study. J dent 2014;11(2):143-14.
8. *Antunes HS, Gominho LF, Andrade-Junior CV, Dessaune-Neto N, Alves FR, Rôças*IN, *et al. Sealing ability* of *two root-end filling materials* in a *bacterial nutrient leakage model*. Int Endod J *2016*;49(10):960–5.
9. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32(2):93-8.
10. Love RM. Enterococcus faecalis–a mechanism for its role in endodontic failure. Int Endod J 2001;34(5):399-405.

11. Xavier CB, Weismann R, de Oliveira MG, Demarco FF, Pozza DH. Root-end filling materials: apical microleakage and marginal adaptation. J Endod 2005;31(7):539-42.
12. Barthel CR, Moshonov J, Shuping G, Orstavik D. Bacterial leakage versus dye leakage in obturated root canals. Int Endod J 1999;32(5):370-5.
13. Tsesis I, Elbahary S, Venezia NB, Rosen E. Bacterial colonization in the apical part of extracted human teeth following root-end resection and filling: a confocal laser scanning microscopy study. Clin Oral Invest 2017;28:1-8.
14. Ma J, Shen Y, Stojicic S, Haapasalo M. Biocompatibility of two novel root repair materials. J Endod 2011;37(6):793-8.
15. Lovato KF, Sedgley CM. Antibacterial activity of endosequence root repair material and proroot MTA against clinical isolates of Enterococcus faecalis. J Endod 2011;37(11):1542-6.
16. Kokate SR, Pawar AM. An *in vitro* comparative stereomicroscopic evaluation of marginal seal between MTA, Glass Inomer Cement & Biodentine as root end filling materials using 1% methylene blue as tracer. Endod 2012;2:36–42.
17. Attik GN, Villat C, Hallay F, Pradelle-Plasse N, Bonnet H, Moreau K, Colon P, Grosgogeat B. In vitro biocompatibility of a dentine substitute cement on human MG63 osteoblasts cells: Biodentine™ versus MTA®. Int Endod J 2014;47(12):1133-41.
18. Shokouhinejad N, Nekoofar MH, Ashoftehyazdi K, Zahraee S, Khoshkhounejad M. Marginal adaptation of new bioceramic materials and mineral trioxide aggregate: a scanning electron microscopy study. Iran Endod J 2014; 9: 144–8.
19. Wang Z, Ma J, Shen Y, Haapasalo M. Acidic pH weakens the microhardness and microstructure of three tricalcium silicate materials. Int Endod J 2015;48(4):323-32.

20. Gomes-Cornélio AL, Rodrigues EM, Salles LP, Mestieri LB, Faria G, Guerreiro-Tanomaru JM, Tanomaru-Filho M. Bioactivity of MTA Plus, Biodentine and an experimental calcium silicate-based cement on human osteoblast-like cells. Int Endod J 2015;50(1):39-47.
21. Moinzadeh AT, Portoles CA, Wismayer PS, Camilleri J. Bioactivity potential of EndoSequence BC RRM putty. J Endod 2016;42(4):615-21.
22. Akcay H, Arslan H, Akcay M, Mese M, Sahin NN: Evaluation of the bond strength of root-end placed mineral trioxide aggregate and Biodentine in the absence/presence of blood contamination. Eur J Dent 2016;10:370-375.
23. Coaguila-Llerena H, Vaisberg A, Velasquez-Huaman Z. In vitro cytotoxicity evaluation of three root-end filling materials in human periodontal ligament fibroblasts. Braz Dent J 2016;27:187-191.
24. Nagesh B, Jeevani E, Sujana V, Damaraju B, Sreeha K, Ramesh P. Scanning electron microscopy (SEM) evaluation of sealing ability of MTA and EndoSequence as root-end filling materials with chitosan and carboxymethyl chitosan (CMC) as retrograde smear layer removing agents. J Conser Dent 2016;19(2):143.
25. Seedat HC, Van der Vyver PJ. An in-vitro comparison of microleakage between three calcium silicate cements and amalgam. S Afr Dent 2016;71(3):100-5.
26. Scelza Mz, Nascimento Jc, Silva Le, Gameiro Vs, De Deus G, Alves G. BiodentineTM is cytocompatible with human primary osteoblasts. Braz oral res 2017;31:e81.
27. Torabinejad M, Rastegar AF, Kettering JD, Ford TR. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. J Endod 1995;21(3):109-12.

28. Fischer EJ, Arens DE, Miller CH. Bacterial leakage of mineral trioxide aggregate as compared with zinc-free amalgam, intermediate restorative material, and Super-EBA as a root-end filling material. J Endod 1998;24(3):176-9.
29. Adamo HL, Buruiana R, Schertzer L, Boylan RJ. A comparison of MTA, Super-EBA, composite and amalgam as root-end filling materials using a bacterial microleakage model. Int Endod J 1999;32(3):197-203.
30. Maltezos C, Glickman GN, Ezzo P, He J. Comparison of the sealing of Resilon, Pro Root MTA, and Super-EBA as root-end filling materials: a bacterial leakage study. J Endod 2006;32(4):324-7.
31. Kazem M, Eghbal MJ, Asgary S. Comparison of bacterial and dye microleakage of different root-end filling materials. Iran Endod J 2010;5(1):17–22.
32. Yildirim T, Er K, Tasdemir T, Tahan E, Buruk K, Serper A. Effect of smear layer and root-end cavity thickness on apical sealing ability of MTA as a root-end filling material: A bacterial leakage study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:e67–72.
33. Nair U, Ghattas S, Saber M, Natera M, Walker C, Pileggi R. A comparative evaluation of the sealing ability of 2 root-end filling materials: an in vitro leakage study using Enterococcus faecalis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112(2):e74-7.
34. Kazem M, Mahjour F, Dianat O, Fallahi S, Jahankhah M. Root-end filling with cement-based materials: an in vitro analysis of bacterial and dye microleakage. Dent Res J 2013;10:46–51.
35. Medeiros PL, Bernardineli N, Cavenago BC, et al. Sealing ability of MTA, CPM, and MBPc as root-end filling materials: a bacterial leakage study. J Appl Oral Sci 2016; 24:148–52.

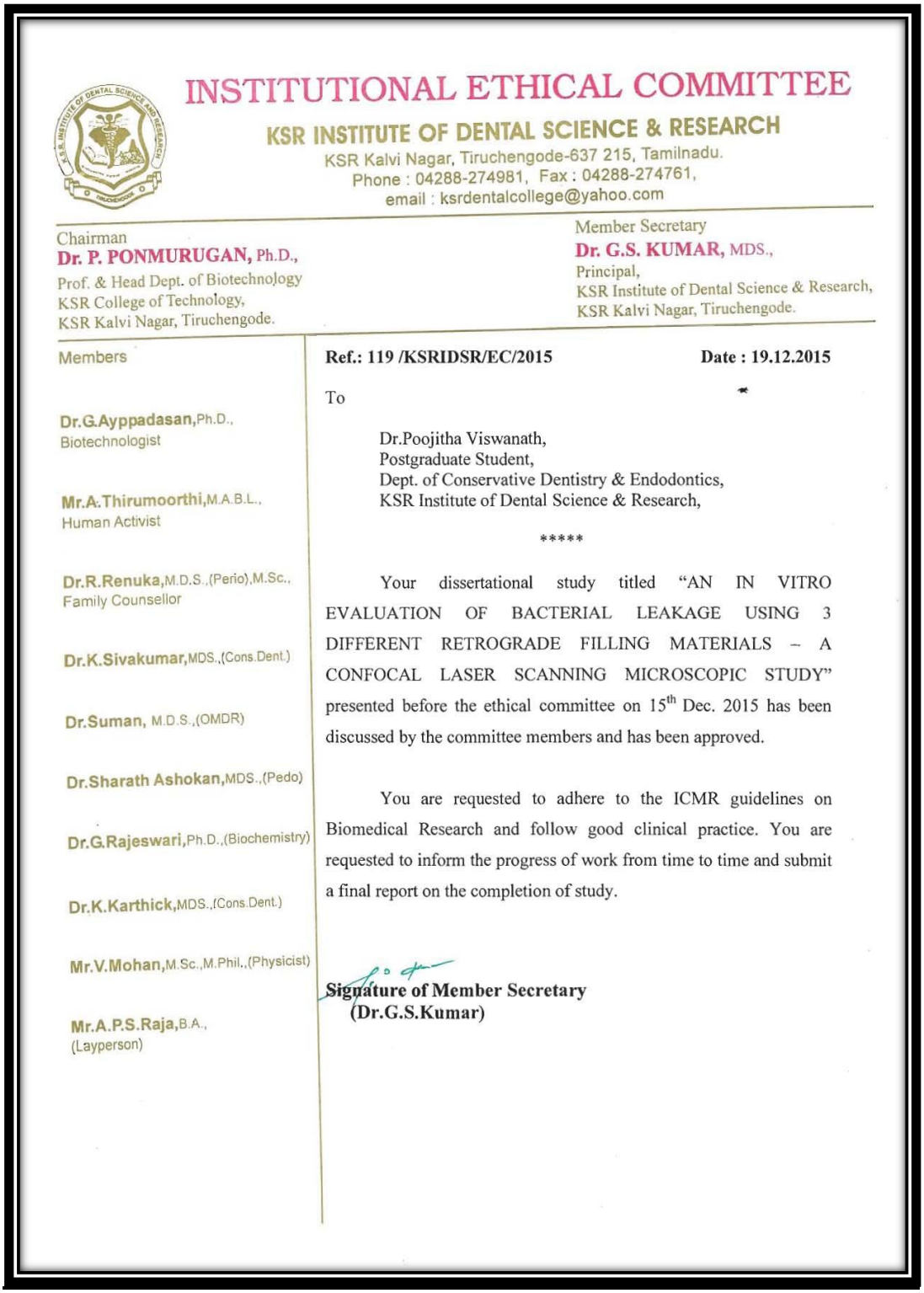
36. Shahariari S., Faramazi F., Alikhani M.Y., Farhadian M., Hendi S.S. Apical sealing ability of mineral trioxide aggregate, intermediate restorative material and calcium enriched mixture cement: a bacterial leakage study. Iran Endod J 2016; 11: 336-340.
37. Eskandarinezhad M, Shahveghar-Asl N, Sharghi R, Shirazi S, Shakouie S, Milani AS. Sealing efficacy of mineral trioxide aggregate with and without nanosilver for root end filling: An in-vitro bacterial leakage study. J Clin Exp Dent 2017;9:e27–33.
38. Ravichandra PV, Vemisetty H, Deepthi K, Reddy SJ, Ramkiran D, Krishna MJ, et al. Comparative evaluation of marginal adaptation of biodentine (TM) and other commonly used root end filling materials – An in vitro study. J Clin Diagn Res 2014;8:243-5.
39. Nanjappa AS, Ponnappa KC, Nanjamma KK, Ponappa MC, Girish S, Nitin A. Sealing ability of three root-end filling materials prepared using an erbium: Yttrium aluminium garnet laser and endosonic tip evaluated by confocal laser scanning microscopy. J Conser Dent 2015;18(4):327.
40. Aziz A, Chandler NP, Hauman CH, Leichter JW, McNaughton A, Tompkins GR. Infection of apical dentin and root-end cavity disinfection. J Endod 2012;38(10):1387-90.
41. Siqueira JF, Rôçcas IN, Abad EC, Castro AJ, Gahyva SM, Favieri A. Ability of three root-end filling materials to prevent bacterial leakage. J Endod 2001;27(11):673-5..
42. Shinbori N, Grama AM, Patel Y, Woodmansey K, He J. Clinical outcome of endodontic microsurgery that uses EndoSequence BC root repair material as the root-end filling material. J Endod 2015;41(5):607-12.
43. Camilleri J, Grech L, Galea K, Keir D, Fenech M, Formosa L, Damidot D, Mallia B. Porosity and root dentine to material interface assessment of calcium silicate-based root-end filling materials. Clin Oral inves 2014;18(5):1437-46.

44. Zedler, Amber. (2016). Dimensional Changes of ProRoot white Mineral Trioxide Aggregate, EndoSequence Root Repair Material, and Biodentine During Setting Using Digital Image Correlation. Retrieved from the University of Minnesota Digital Conservancy

45. Chen I, Karabucak B, Wang C, Wang HG, Koyama E, Kohli MR, Nah HD, Kim S. Healing after root-end microsurgery by using mineral trioxide aggregate and a new calcium silicate–based bioceramic material as root-end filling materials in dogs. J Endod 2015;41(3):389-99.

ANNEXURE

APPENDIX I



INSTITUTIONAL ETHICAL COMMITTEE

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To

Dr.Poojitha Viswanath,
Postgraduate Student,
Dept. of Conservative Dentistry & Endodontics,
KSR Institute of Dental Science & Research,

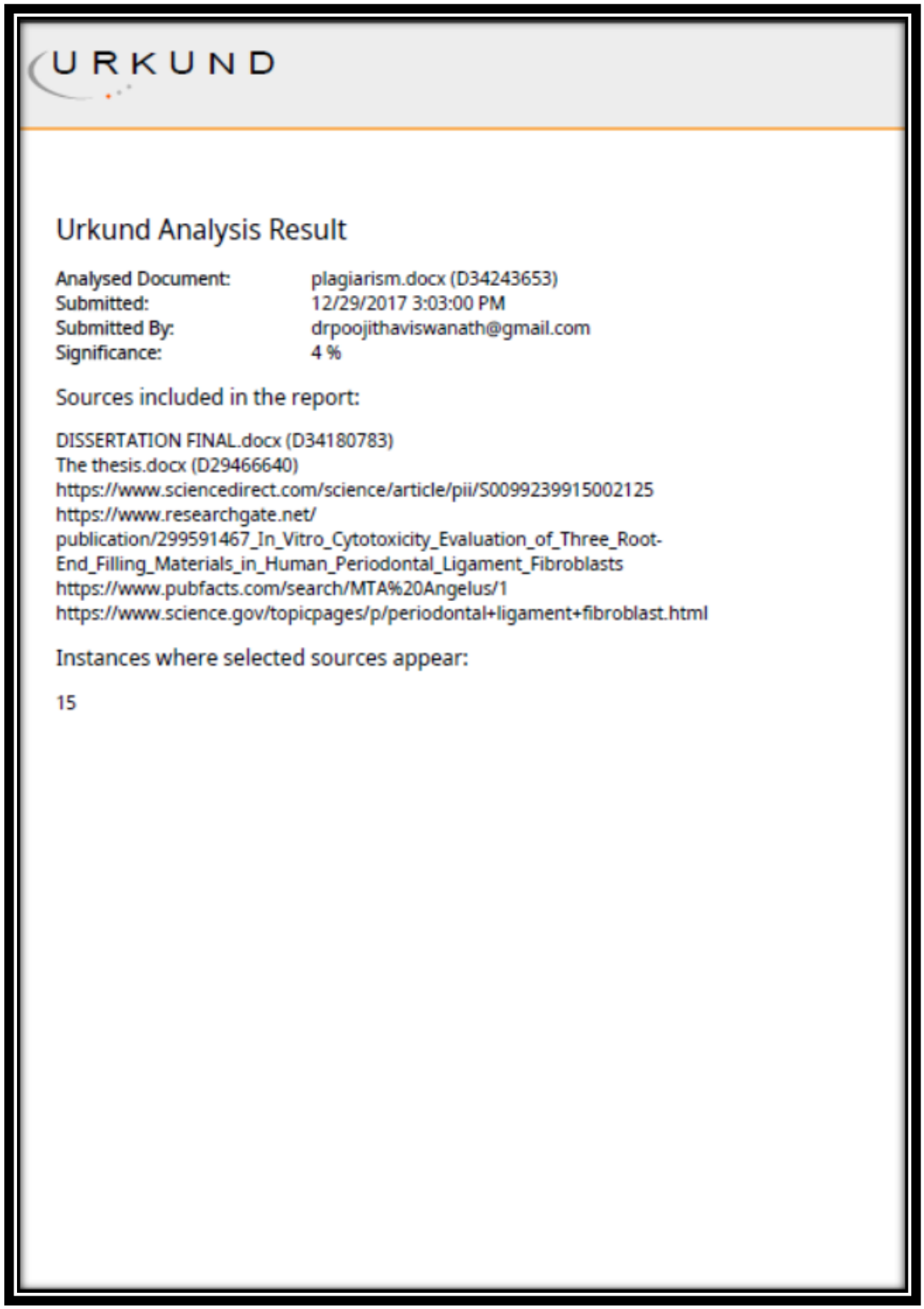
*****

Your dissertational study titled "AN IN VITRO EVALUATION OF BACTERIAL LEAKAGE USING 3 DIFFERENT RETROGRADE FILLING MATERIALS – A CONFOCAL LASER SCANNING MICROSCOPIC STUDY" presented before the ethical committee on 15th Dec. 2015 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.

Signature of Member Secretary
(Dr.G.S.Kumar)

APPENDIX II



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APPENDIX III

CERTIFICATE - II

This is to certify that this dissertation work titled "**An in vitro evaluation of bacterial leakage using 3 different retrograde filling materials - A confocal laser scanning microscopic study"** of the candidate **Dr.Poojitha Viswanath** with registration number **241517403** for the award of "**Master of Dental Surgery"** in the branch of **Conservative Dentistry and Endodontics** . I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **4 %** percentage of plagiarism in the dissertation.

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